HEALTH AND PRODUCTIVITY MONITORING OF CAGE-CULTURED ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS*)

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that the thesis is acceptable in form and content, and that a satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on June 28th 2012.

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Date June 28 2012

ABSTRACT

The Atlantic Canadian aquaculture industry is dominated by Atlantic salmon production. In recent years, infectious disease, parasitic infestations, and price fluctuations from international competition have caused disruptions to the industry. Diversification of the industry away from Atlantic salmon production is a potential strategy to insulate the industry from these fish health and market challenges. Atlantic halibut has long been a primary candidate for this diversification. However, the early commercialization of the species over the last 15 years has failed to reach its potential, owning primarily to a lack of information on the biology of the species, best management practices and proven economic feasibility.

To address this information gap, a multi-objective Randomized Controlled Trial (RCT) was conducted at a commercial farm on the Bay of Fundy in New Brunswick. The study collected detailed information on the growth and survival of 5000 Atlantic halibut individually identified with Passive Integrated Transponder (PIT) tags and followed over a four year grow-out period.

The main objectives of this research were: (1) to evaluate the impact of individual fish characteristics on growth, survival and farm economics, (2) to determine the effects of oil-adjuvanted vaccines on growth performance, survival and vaccine associated lesions, (3) to evaluate the suitability of FT4 Lock-on tags as an external tagging method for individual identification of Atlantic halibut, and (4) to test a Stratified Transport System (STS) as a means of improving fish welfare and the economics of overland halibut transport.

A variety of fish level characteristics were found to be important predictors of productivity. Identifying and culling fish with these specific characteristics prior to grow-out was identified as a method to improve overall farm productivity. The side-effects of oil-adjuvanted vaccines were found to be mild in Atlantic halibut, thereby identifying oil-adjuvants as an available tool for future vaccine development. FT4 Lock-on tags were found to be suitable for identifying cage-cultured halibut with the exception of substantial impacts on growth. The STS was demonstrated to reduce post-transport mortality, establishing it as a cost-effective transport solution over currently practiced methods.

In conclusion, this research allows producers to make evidence-based management decisions, to strengthen and facilitate the continued development of the Atlantic halibut aquaculture sector in Atlantic Canada.

DEDICATION

I dedicate this work to fish farmers everywhere. Without their tireless efforts, against all odds, there would be less to eat and more to complain about.

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
AIC	Akaike Information Criterion
BCR	Benefit-cost ratio
CI	Confidence interval
DD	°C-day
DFO	Department of Fisheries and Oceans
FT4	Floy lock-on tag
FAO	Food and Agriculture Organization
FCE	Food conversion efficiency
FCR	Food conversion ratio
g	Gram
i.p.	Intraperitoneal
kg	Kilogram
L_F	Fork Length
l	Litre
m	Metre
mg	Milligram
mt	Metric tonne
n	An indefinite number
NPV	Net present value
P	*P*-value
P_{corr}	*P*-value with Bonferroni correction
PIT	Passive integrated transponder

P	Proportional increase in weight
SD	Standard deviation
SE	Standard error
TMS	Tricaine methanesulfanate
UTS	Unstratified transport system
RCT	Randomized controlled trial
RPS	Relative percent survival
G	Specific growth rate
STS	Stratified transport system
W	Weight
W_0	Initial weight

Chapter I: GENERAL INTRODUCTION

1.1 Introduction

At their current state fisheries face uncertain futures, with predictions indicating the global collapse of all current fisheries by 2048 (Worm et al. 2006). The United Nations predicts the global population to grow from 7 billion today to 9.3 billion in 2050 (Lee 2011). This growth coupled with a wider understanding of the health benefits associated with marine protein sources (Mitchell 2011) can only increase global demand for these products intensifying pressure on our already compromised ocean resources (FAO 2010).

Aquaculture is an ancient activity and has been practised for thousands of years; however, following World War II its growth has been unprecedented. This is mainly attributed to its unparallelled ability to efficiently use scarce and valuable resources while producing highly valued products. The growth of aquaculture is unmatched by any other animal production industry both in terms of value and production (FAO 2010), and the per capita consumption of aquaculture products has increased 6.9% annually from 1970 to 2006. This continued growth has recently allowed aquaculture to reach the unique milestone of producing half of all the fish and shellfish for human consumption (Naylor et al. 2009; FAO 2010). These impressive figures highlight the intense growth of global aquaculture, but also the declining state of capture fisheries.

In Canada, specifically Atlantic Canada, the climate, market demands, and prices have enabled finfish production, primarily Atlantic salmon (*Salmo salar*) net pen

aquaculture, to grow. Atlantic salmon production is greatest in terms of both volume and value for Canadian aquaculture (Government of Canada 2011).

1.2 Diversification of aquaculture

Due to their biological nature and market dependency, aquaculture investments are subject to many unforeseeable elements. The industry in Atlantic Canada is dominated by a single species (Atlantic salmon) for domestic consumption and export to the USA. Production is geographically concentrated in New Brunswick, with recent expansions of the industry to insular Newfoundland and Nova Scotia. Salmon is a globally traded commodity and, overall, Atlantic Canada is a relatively small producer (Ridler et al. 2007). With multiple nations involved, prices tend to fluctuate and factors well beyond the control of local producers can affect individual enterprises (Zarnikow 2010). For this reason, the Atlantic Canadian aquaculture industry and the rural economies that depend on it, face considerable risk should prices fall (Ridler et al. 2007). Diversification of the industry into other species can help to moderate these economic risks.

As with most animal farming activities, disease issues begin to appear following the intensification and commercialization of aquaculture (Bondad-Reantaso et al. 2005). Disease prevention is vital to sustain aquaculture production and protect wild fish populations (Harikrishnan et al. 2010). Diversification of the industry from one species to alternative species can introduce biosecurity barriers due to differences in pathogen susceptibility. The Atlantic salmon industry has faced a variety of fish health challenges that continue to constrain the industry. The most notable and economically important

examples are Infectious Salmon Anemia (ISA), caused by an orthomyxovirus that is often lethal to the salmon and economically damaging to the producer (Falk et al. 1997), and ectoparasitic infections with sea lice (*Lepeophtheirus salmonis*) (Westcott et al. 2004).

1.2.1 Considerations for choosing a candidate aquaculture species

The following eight considerations are important when selecting a new aquaculture species (Le François et al. 2002)

- Closed life cycle
- Available broodstock
- Biology and behaviour amenable to commercial production
- Biological requirements can be met
- Growth
- Disease resistance
- Market
- Profitability

The ability to breed and grow a species under commercial culture conditions and characteristics of biology and behaviour that are amenable to the stresses associated with commercial culture are important considerations. Sites must meet the biological requirements of the species (temperature, salinity, water quality, etc.). Rapid growth results in shorter time to market and greater financial returns on initial investments while

reducing the period of risk for disease occurrence (Le François et al. 2002). However, growth must be paired with efficiency so that feed inputs are optimally assimilated into marketable product. Of the three productivity measures, efficiency is the most difficult to quantify but represents a significant impact (Imsland et al. 2010) since feed costs are the largest component cost of production.

The pre-existence of a capture fishery for the same species is usually a good predictor that the same species raised in aquaculture already has an established market. Fishery products were traditionally consumed close to point of landing because of spoilage issues; over time this leads to local product knowledge and a developed market. Traditional catches of Atlantic salmon, Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), and Atlantic halibut (*Hippoglossus hippoglossus*), are common on Canada's east coast. The unfortunate reality is that a declining or collapsed traditional fishery is favourable to the successful development of a new aquaculture species. Capture fisheries are the largest contributor to market instability. Finfish aquaculture operations cannot compete with a viable commercial fishery in a price-driven market. The absence of an Atlantic salmon fishery is one of the reasons Atlantic salmon aquaculture has thrived.

Atlantic cod, haddock, and Atlantic halibut are considered potential alternatives to Atlantic salmon based on the fact that the biological requirements for farming all three species are similar. The entire life cycle of all three species can occur in captivity. Atlantic cod aquaculture is hindered by early maturation and a limited base of health management knowledge (Rosenlund & Halldorsson 2007). In addition, profitability is affected by market price fluctuations caused by substitute species and variable supply

(Asche et al. 2009). Haddock culture has been plagued with many challenges which include; difficulty maintaining healthy broodstock (Martin-Robichaud 2003), high levels of mortality during grow-out (Frantsi et al. 2002) and a healthy local commercial fishery (TRAC 2011). Atlantic halibut has a small but stable seasonal fishery in Atlantic Canada, producing approximately 2000mt annually (DFO 2011a; DFO 2011b) with a short season and small volumes that fail to satisfy market demand. There is a much larger capture fishery for Pacific halibut (*Hippoglossus stenolepis*) on the west coast of North America that seasonally affects the demand and market price for cultured Atlantic halibut. However, the Pacific halibut fishery is seasonal and Atlantic halibut enjoys a price premium (BeiBei et al. 2008). Atlantic halibut have a comparatively high market price, proximity to lucrative east coast markets and no known susceptibility to ISA or sea lice, making it a good candidate to diversify the aquaculture industry in Atlantic Canada.

1.3 Atlantic halibut

The genus *Hippoglossus* is translated from the Greek words "Hippo" (horse) and "glossus" (tongue). Atlantic and Pacific halibut were long thought to be the same species but in 1904 Russian ichthyologist P.J. Schmit, noted that Pacific halibut (*H. stenolepis*) have differently shaped scales and slight differences in pectoral fin length and body shape. For this reason, the species were distinguished from one another and the Pacific's given the species name *stenolepis*, "steno" meaning narrow and "lepis" meaning scale.

The Atlantic halibut (*H. hippoglossus)* is the largest of the asymmetric flat fishes belonging to the family Pleuronectidae (the right eyed flounders) and can reach sizes of over 300 kg (Bromage et al. 2000). Two major characteristics highlight this asymmetry, the first being that both eyes are located on one side of the head. This is most often the right side of the head (dextral; hence 'right–eyed') and results in two unique sides to the fish, the ocular side (eyed side) and the abocular side (blind side). The second most notable characteristic of halibut asymmetry is the differential colouration. The blind side is completely white, a stark contrast to the ocular side that is darkly pigmented with black, brown and yellow hues. In younger individuals, a specific white-dark pattern can be observed on the ocular side. This camouflage pattern includes a white collar just posterior to the operculum and 6-9 small white rosettes along the outer edges of the body, and is presented when the fish are concealed by the surrounding environment while lying on bottom. This pattern quickly darkens and disappears if the fish moves into the water column or becomes stressed (John Bailey Pers. Comm.).

Halibut are known for their tremendous growth rate, which becomes most apparent at ages greater than 10 years (McCracken 1958) when their size allows access to larger prey items. Sexual dimorphism is evident, with males maturing earlier (3-5 years) and at a smaller size (1-4kg) than females (7-9 years, 12kg), both in culture and in the wild (Bjornsson 1995; Roth et al. 2007). Juvenile halibut are voracious eaters until they reach approximately 30cm in length; this has important considerations for aquaculture management. During this period, fish must be adequately fed to prevent cannibalistic behaviour and physical damage to eyes, pectoral fins and tails (Greaves & Tuene 2001). As wild Atlantic halibut reach 30 to 80cm their diet expands to include

other fishes, and once past 80cm their diet consists exclusively of other fishes (Kohler 1967). Atlantic halibut spawn in late winter or early spring, mainly February to April in most of the Canadian range (McCracken 1958; Kohler 1967).

1.3.1 Development of halibut culture in Atlantic Canada

The potential for Atlantic halibut as an alternative to Atlantic salmon aquaculture is being evaluated in Atlantic Canada. Atlantic halibut are considered a good candidate for a variety of reasons which include:

- Adaptable to farm conditions
- Closed life cycle
- Efficiently convert food into marketable flesh
- Resistant to common marine pathogens and parasites (particularly those affecting Atlantic salmon)
- A firm, white, mild tasting flesh with good shelf life
- High product value

The development of the halibut aquaculture industry has been slow, mainly due to high investment costs and a long grow-out cycle. Current sea cage production can grow a market weight fish (4-5kg) from the juvenile stage (200-400g) in 42-48 months. Shortening the production cycle, central to improving the profitability of halibut farming, could be achieved by increasing individual fish performance with selective breeding programs. The high cost of juveniles ($12-16 CAD per 200-400g fish) is major factor affecting the profitability of halibut culture (Brown 2010; Sykes et al. 2012). High

stocking costs require greater investments that must be carried through the entire production cycle. Unproven culture methods and limited knowledge of the risks to production make investors wary. This is especially the case for marine cage-based operations which are open to the environment and exposed to considerably more unpredictable influences (e.g., pathogens, predators, and weather). The normal habitat of wild Atlantic halibut is not in areas where aquaculture sites would normally be located, contrasted with the annual migrations patterns of wild Atlantic salmon traveling in close proximity to cage farms when returning to their native rivers. This natural separation may be advantageous in preventing the transmission of pathogens from wild halibut stocks to aquaculture stocks and *vice versa*.

The number of locations available for the development of halibut cage-culture in Atlantic Canada is limited. New Brunswick is the most likely location, but a moratorium on the development of new cage sites will make this difficult. There are currently 96 protected inshore site leases in the Bay of Fundy in New Brunswick, mainly used for salmon. Biomass restrictions result in limits on farm sizes. With the expansion of the industry, these restricted sizes may be uneconomical for salmon production. Halibut, with lower stocking densities, smaller farm sizes and higher final product value could potentially use these sites more economically. Insular Newfoundland has had considerable expansion in its aquaculture industry in recent years. High energy sites and the capacity for larger farms in Newfoundland favour the farming of Atlantic salmon and the development of the regionally significant Atlantic cod rather than Atlantic halibut. A limited number of marine farm sites in Nova Scotia, combined with few suitable protected locations for Atlantic halibut, make Nova Scotia an unlikely location

for halibut cage-culture. The shallow unprotected waters that surround Prince Edward Island, seasonally experience lethal temperature extremes and harsh winter sea ice, resulting in poor conditions for sea cage siting.

Despite the availability of hatchery expertise and capacity to reliably produce juveniles in Atlantic Canada, the industry has remained on the brink of commercial development for almost 20 years. As mentioned, the limited investment, high costs of stocking, and converting farms, and a long production cycle currently limit the development of the Atlantic halibut industry in Atlantic Canada (Table.1.1). The key areas of profit improvement for halibut culture include reducing the price of juveniles, which would lower upfront financing costs and the overall financial risk and shortening the entire production cycle from approximately 60 months from egg to plate to 36 months. This would be more in line with the Atlantic salmon production cycle and represents a reasonable period of time which farmers can be expected to operate with negative cash flows. Lastly, without at least some history regarding disease risks to sea cage-culture, financial investment will be limited (Forster 1999).

Table 1.1 Economic and production considerations related to farming Atlantic halibut (*H. hippoglossus)* and Atlantic salmon (*S. salar).* Due to the smaller market volumes, area demanding nature of the Atlantic halibut, high cost of juveniles and high end product value, the size of individual halibut farms is likely to be smaller than traditional Atlantic salmon farms. The monetary figures reflect the volumes of traditional lease that could accommodate sixteen,70m Polar Circle sea cages stocked with 400g halibut juveniles.

	 *H. hippoglossus*	 *S. salar*
Juvenile cost	**$16**	**$2**
Grow out period	**5 yr**	**3 yr**
Farm populations	**84,000**	**240,000**
End product value	**$7/lb** (HOG)	**$2.5-3.5/lb** (HOG)
Stocking cost	**$1.4 million**	**$0.5 million**
Farm Gate Value	**$5.9 million**	**$6.0 million**
Risk	**Unproven / Unknown risk**	**Proven/ Established Risk**

1.4 Atlantic halibut production

The two main stages of halibut aquaculture are the hatchery and the on-growing stages. Hatcheries are typically land-based recirculation facilities. Halibut are iteroparous serial spawners. This characteristic, in combination with a late age of maturity and the ability to spawn for multiple years, mean mature broodstock are retained for multiple years, thus preventing lethal health sampling procedures. Female Atlantic halibut grow more rapidly and sexually mature later than males, making them economically more favourable to culture. The production of all-female populations can be accomplished by breeding sex-reversed males to normal females (Hendry et al. 2003) and is a strategy currently under evaluation to shorten grow-out periods (Gerald Johnson Pers. Comm.). Out-of-season spawning is now achieved through daylight and temperature manipulation, allowing a year round supply of juveniles (Brown 2010). Compared to salmonids, the eggs of halibut and other marine fish require special care because they are small and delicate. Replicating the cold, dark, and stable environment found in the mesopelagic zone of the ocean where halibut embryos and larva develop is challenging.

Like other fish, Atlantic halibut start life as a symmetrical fish (Fig. 1.1). Metamorphosis occurs when one eye (typically the left eye) migrates to the other side, resulting in an asymmetric flatfish. The process of metamorphosis is developmentally complicated and its timing depends largely on larval growth rates. Modification of lighting and feeding strategies to slow the gut transit time recently has been documented to improve eye migration success (Harboe et al. 2009), which has long been a problem

in commercial culture (Hamre et al. 2007). Eye migration complications are rarely observed in the wild (Chabot & Miller 2007). Following metamorphosis, the juveniles are weaned from live feeds onto formulated feeds and then typically moved to shallow nursery tanks at which time temperatures are increased for optimal growth and regular feedings to occur. Fish grow from less than 1g up to 400 grams during the nursery stage. During this time, fish are regularly sorted and graded according to size and quality at which time intraperitoneal (i.p.) vaccination can take place.

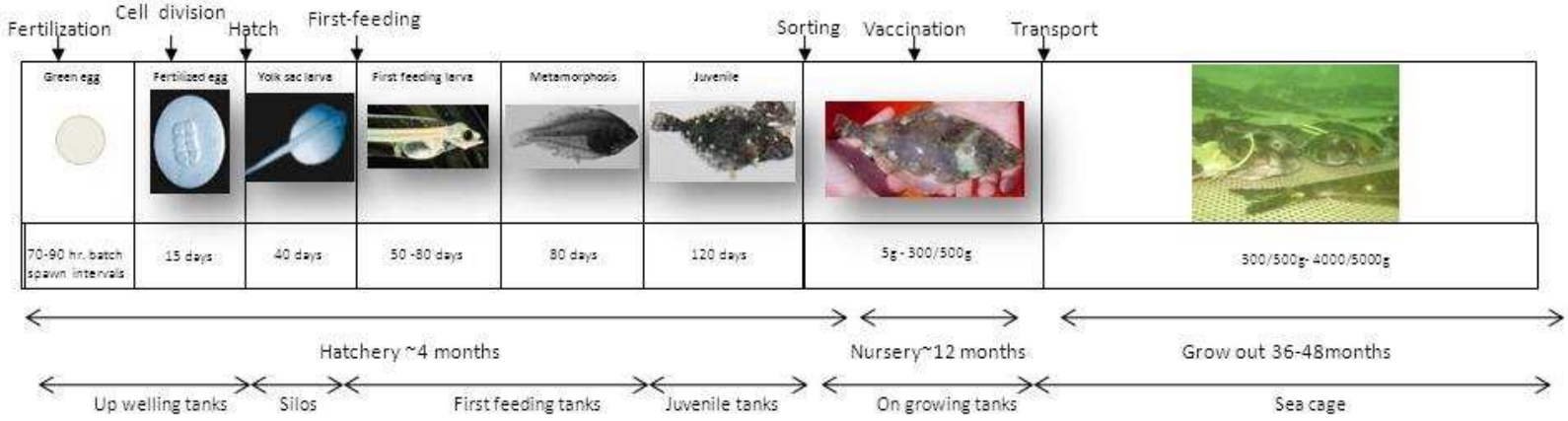

Fig. 1.1 Production cycle of Atlantic halibut (Hippoglossus hippoglossus). Early rearing times recreated from Brown (2010), photos from Scotian Halibut Ltd.

Fish are transported overland to grow-out sites when they reach approximately 200-400g. The transport protocols for Atlantic halibut have been developed almost exclusively through industry experience (Brown 2002) and by transfer of knowledge gained from the extensive transport of salmonids. During transport, halibut settle on top of one another resulting in aggregations of halibut several layers deep. This can impede the exchange of water around the fish, creating heterogeneous areas of suboptimal conditions or "dead spots" (Brown 2002; Reig et al. 2007). Equipment designed for the transport of Atlantic salmon has led to variable success when moving halibut juveniles, including suboptimal stocking densities and variable post-transport mortality. The high cost and limited availability of halibut juveniles make any post-transfer mortality economically important.

1.4.1 Atlantic halibut production methods

Atlantic halibut production takes place in seawater aquaculture facilities, such as land-based facilities (flow-through/recirculation), tidal lobster pounds or modified sea cages (Fig 1.2) (Brown 2002). Land-based recirculation facilities have a high degree of control for a variety of factors ranging from water temperature to biosecurity. However, these advantages come with considerable infrastructure and operating cost associated with land, buildings and energy. Cage-based-culture has long been considered the only economically viable method to raise Atlantic salmon at its current economies of scale (Mortensen et al. 2007). The siting of halibut cage farms requires special consideration. Protected low energy sites are preferred for halibut which spend a large proportion of

time settled on the cage bottom, and in rough conditions the continual motion of the cage bottom disturbs the fish (Martinez Cordero et al. 1994).

Atlantic halibut, like other flatfishes, require surface area on which to settle. This is in contrast to Atlantic salmon which use most of the water column, greatly increasing the number of fish that can be reared in a production unit. Land-based facilities typically have rigid walled tanks, allowing the addition of shelving to increase the surface area within tanks. Sea cages lack rigid structure so the addition of shelving is more difficult and, before doing so, it must be considered how shelving may be affected by tides, rough weather and bio-fouling.

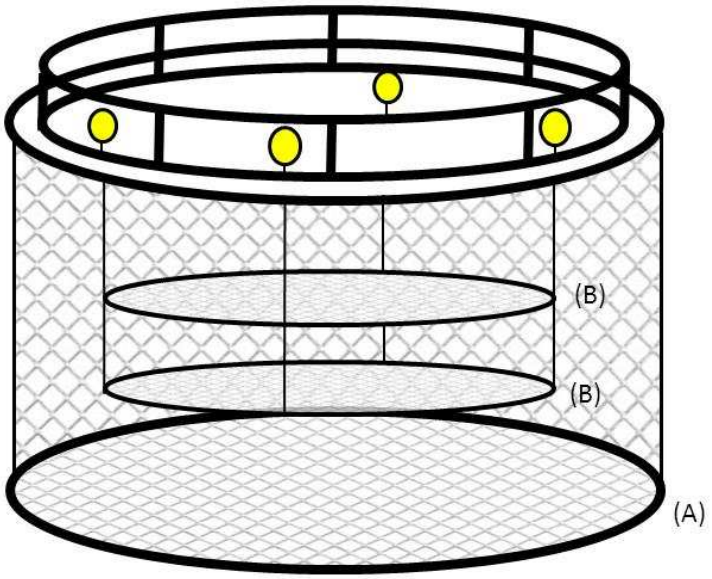

Fig.1.2. A traditional sea cage used for salmon production that has been modified with a flat bottom net tensioned using a weighted sand ring (A) to provide surface area for settlement. Cages are often outfitted with one or more shelving units (B) to increase the surface area for the growing Atlantic halibut to settle.

1.5 Research in aquaculture

Aquaculture is a new industry compared to most other food animal production industries. Veterinarians and fish health professionals are often presented with cases for which limited information exists or comparable information is only available for a different species or culture conditions. Commercial controlled trials allow veterinarians to respond to health problems while building evidence to improve the health of farmed fish. With the expansion of the industry, both in terms of size and diversity, there is an ever expanding requirement for evidence-based medicine.

1.5.1 Randomized controlled trials

When no previous evidence is available, randomized Controlled Trials (RCTs) are considered the most rigorous tool that an epidemiologist can use to provide evidence for animal health interventions and to monitor animal productivity (Dohoo et al. 2009). Properly designed and implemented field trials can satisfy the requirement of external validity for veterinarians and fish health personnel attempting to establish answers to natural disease in production settings. Whereas laboratory based trials are cost and time efficient, allowing completion with unparalleled internal validity, they take place in unnatural settings and, by design, compromise the external validity of study findings. This leaves researchers uncertain of the applicability of findings to natural populations in the 'real world' (Martin 1989). Disease requires the combination of host, pathogen, and environment (Snieszko 1973). Under laboratory conditions, fish can be challenged with disease agents through a variety of methods (i.p. and i.m. injection, bath and cohabitation) (Nordmo & Ramstad 1997). However, complex dynamics between the

host, pathogen and the environment are responsible for subtle and sometimes dramatic differences when comparing the results of laboratory and field studies (Aunsmo et al. 2008; Martin 1989).

1.5.2 The art and science of randomized controlled trials

The nature of the aquaculture industry creates a number of difficulties in the design and implementation of RCTs. Although conducting RCTs is a scientific task requiring a combination of statistical, biological and medical skills, it is both an 'art' and a 'science'. Dealing with people is the 'art', as valid RCT completion is unmanageable without the cooperation of industry partners, production staff and technical field crews (Mitchell 1997). Commercial trials strive for laboratory precision in what are rather unpredictable environments, requiring the collaboration of personnel having expertise not often found in laboratories.

Studying infectious disease in commercial settings can be difficult as the study population may never be exposed to the disease of interest, as commercial producers are understandingly unwilling to knowingly introduce pathogens. Studies employing multiple cages can reduce this risk by increasing the chance of exposure, but this comes with considerable cost. Although fish-level studies within one tank are an effective way to minimize tank effect, the scale of a commercial research trial becomes costly and tank/cage replicates are typically restricted in numbers.

1.5.3 Statistical considerations for randomized controlled trials

Appropriate statistical assessments are critical when conducting and designing successful field trials. The three pillars of a control trial include establishing a defined outcome measure, preventing bias and considering the role of chance (Ribble 1989).

The role of chance, also referred to as the internal validity of the study, reflects whether an observed difference between treatment groups is true rather than due to random error. A high degree of internal validity eliminates bias and error from playing a role in a trial (Martin 1989).

Bias occurs when a factor other than the risk factor of interest or treatment, systematically distorts a measured outcome, causing a difference between the groups that is outside a random variation (or error). Sampling procedures are a common source of bias in commercial aquaculture field trials (Hopkins & Yakupitiyage 1991). Bias can present itself at any one of four key times: during selection of the animals for inclusion in the study, during follow up and treatment, during measurement of the outcome, and during final analysis where preconceived ideas may result in improper decisions (Hammell 1992). Blinding the investigators and study personnel is the most appropriate way to avoid many of these biases in RCTs (Dohoo et al. 2009).

Obtaining sufficient sample size is a central problem to the completion of multivariate aquaculture research (Nortvedt & Tuene 1998). Sample size decisions must be given adequate attention when designing any RCT. In general, sample size considers: 1) the variability in the outcome measure among individuals (regardless of treatment group), 2) precision of the estimates required, 3) the level of confidence that a concluded

difference is not due to chance, 4) logistics of sampling, and 5) the cost of sampling. When conducting individual fish-level studies, sample size is rarely a limiting factor. In RCTs sample size is most often limited by the number of fish that can be physically sampled, given the time, space and personnel restrictions. This permits investigators to observe small differences in treatment groups which may have economic importance when scaled to farm or industry outputs.

Fish lost to follow up, particularly unrecorded mortalities, can have substantial impacts on study outcomes. In aquaculture RCTs, many factors contribute to this occurrence, including high summer temperatures causing rapid decomposition of mortalities, cannibalism, animal (or human) depredation, escapees, and counting errors on original stocking numbers (Puebla et al. 2000). These losses commonly range from two to five percent, but can occasionally be much higher (Puebla et al. 2000).

1.5.4 The unit of concern

In RCTs the unit of concern is the level at which treatments are applied to subjects. Five units of concern are possible in commercial aquaculture settings. 1) Region or country –This level is rarely used because of the difficulties in obtaining replication of units for randomization and blinding. 2) Individual farm sites – Although site level randomization and blinding are possible, this level presents other challenges. Sites are often under different ownership and have different management practices. Therefore, using multiple sites introduces additional factors and uncertainty which must be considered. The geographical separation of farms also increases cost and logistical difficulties for researchers. The treatment (or comparison) must be randomly allocated at

the site level and this may not be agreeable to the farm management. 3) Production unit (i.e., tank, pond, cage) – This level is the most commonly employed unit of concern in aquaculture. Production units are often numbered and are conveniently clustered for the procedures of the trial. Multiple units allow the replication of treatments within a single farm or within a few farms thereby controlling additional sources of variability. When conducting studies using production units as a unit of concern, a 'cage' or 'tank effect' may affect the results (Speare et al. 1995) making it important to distribute such effects randomly or in some controlled fashion. 4) Fish-level – studies in which treatments are assigned to individual fish represent the smallest unit of concern for most RCTs. However, this requires the identification of individual fish, which can be onerous. Fish-level trials allow all the study units to be located in one tank, thereby removing 'tank' or 'group effect' as a potential confounder. Fish-level data provide greater replication, thus permitting the use of robust statistical procedures based on large sample sizes (Aunsmo et al. 2008; Burnley et al. 2010). Individual fish studies are rare in aquaculture due to the cost and time required to identify and measure individuals. So uncommon in fact, that they are only very briefly mentioned in a recent text book dedicated to aquaculture statistics (Bhujel 2009). The majority of marine aquaculture trials use a group or cage of fish as the unit of concern. Individual fish studies allow investigators to study multiple factors at once, a useful feature for hypothesis generation. However, it is important to recognize that as more outcomes are considered statistically significant differences are more likely to be detected by chance. 5) Tissue/organ level – the one exception where individual fish may not be the smallest unit of concern is when multiple features are

studied on a single fish, e.g., a recent eye-level study on Atlantic halibut (Treasurer et al. 2007).

1.5.5 Choosing an outcome

In commercial aquaculture, productivity measures are reflective of profitability. Using productivity measures as outcomes in RCTs has advantages for practical interpretation compared to other measures such as biochemical and physiological measurements. For example, in a study investigating optimal transport densities for winter flounder (*Pseudopleuronectes americanus*), the fish were subjected to different transport densities while being shipped from hatcheries to release sites. The results determined statistically significant differences in cortisol levels between the treatment groups at the time of release (Sulikowski et al. 2006). Despite a clear and appropriate statistical analysis, researchers could only speculate that the difference between the two groups was biologically important and likely to influence survival following release in the wild. Choosing productivity outcomes as measures in RCTs automatically infers biological or economic relevance.

Researchers are likely to have greater adoption of research outcomes in the industry when using productivity outcomes because the research provides evidence that is more applicable to the industry in metrics that can be interpreted both biologically and economically. There are three outcomes that are of primary concern to the economic performance of the aquaculture producer; growth, efficiency and, survival. These three metrics can be grouped under the umbrella of productivity.

Growth is an important metric to establishing production times and harvest sizes. During the life of a fish there are natural changes in growth rate, referred to as growth stanzas. Commonly used in aquaculture studies, growth is measurable at both the individual fish and the sea cage level. Growth can be determined simply by finding the change in weight between two time periods or by calculating the specific growth rate (G) or the proportional increase in weight (Pr). To find growth at the individual fish level, individual fish identification is required.

Efficiency is less commonly used in RCTs because it is difficult to measure. The most often used measure of efficiency is the food conversion ratio (FCR) also known as food conversion efficiency (FCE). It is simply the ratio of weight gained over the mass of the feed consumed. Individual FCRs are not calculated in most trials because of the difficulty to determine individual fish consumption. Crude approximations of FCR can be obtained at the cage level by using the total biomass increase over the total mass of feed fed. A cage-level measure cannot be compared statistically in an individual fish-level study and so a measure of efficiency was not possible for the halibut study reported in this thesis.

Survival is the third most commonly used productivity measure. Cumulative survival (or conversely mortality) provides a single summary statistic for a group. This can be more informative when time of mortality can be specified for each individual, and is appropriately called time to event (i.e., death) data. Incorporation of a time component into mortality is important, because the impact of mortality on productivity is time sensitive. The longer the fish lives on the farm, the more feed is consumed resulting in a higher cost of each mortality. Said another way, mortality occurring early in

production is less of a financial loss compared to mortality occurring later in the production cycle. Cumulative measures that cover the entire production cycle fail to provide this information.

Choosing an outcome is a critical component of study design and should be strictly adhered to by the investigator. Outcomes should be objective and at least one outcome should be a measure of productivity, so that the applicability of a treatment can be evaluated. For example, a vaccine with impressive protective effects may appear advantageous, but if that vaccine causes side-effects of reduced growth and abdominal adhesions that result in carcass down grading at harvest, the overall benefits of that vaccine are drastically diminished.

It is also important that outcomes be well defined. In studies with too many outcomes, investigators run the risk of finding statistical significant differences among treatments that result from little more than chance.

1.5.6 Economic implications of fish trials

Calculating and understanding the economic impact of scientific trials is an important component of control trials, particularly those involving alternative aquaculture species where the financial viability of such operations can be tenuous. Control trials often have very specific and critical questions, however interpreting those outcomes and putting them in a format that is understandable by the end users is important. In the case of RCTs applied to commercial scale aquaculture, the end users are producers and their veterinarians, funding agencies and researchers. For these groups, economic interpretation is important. Where appropriate, we have used common

economic tools to make the results of this study more readily available to end users by providing economic interpretation of study outcomes.

1.6 Thesis objectives

The overall objective of this field study was to provide insight into the health and productivity of cage-cultured Atlantic halibut in the Bay of Fundy. Developing improved health management decisions for this alternative aquaculture species in Atlantic Canada was an end goal of the project. Four separate studies, each with the goal of improving the health and productivity of cage-cultured Atlantic halibut, were undertaken and described within the thesis.

1.6.1 Abnormalities and Malformations

Developmental malformations remain an unwelcome but unavoidable aspect of commercial aquaculture production. Malformed fish appear in highly variable and unpredictable numbers in farmed fish. The culture of Atlantic halibut is particularly plagued by this problem due to their complicated development and specific rearing requirements.

> *"High numbers of malformed individual fish can cause severe financial losses for small and medium-size enterprises" – Federation of European Aquaculture Producers – FINEFISH program*

In addition to decreased growth performance, malformed fish create marketing difficulties resulting in decreased product value. Malformed fish are also suggested to be a nidus for disease within a production cohort, potentially compromising the farm.

Malformations are recognized as a major constraint to the aquaculture industry, particularly newly emerging marine species such as halibut. However, despite their importance, very little data have been published on the impact of malformations on productivity of farmed fish.

A complicated larval development, combined with stringent rearing requirements that are difficult to replicate at commercial scales make developmental malformations a relatively common occurrence in flatfish culture. Malformations can result from improper environmental conditions (e.g., tank hydrodynamics, temperature, photoperiod, tank colour) (Mangor-Jensen et al. 1998; Yamanome et al. 2005), nutritional deficiencies (e.g. nutritional composition, density of live feeds in tanks, and feed size) (Hamre et al. 2005; Harboe et al. 2009), or parental genetics (Paperna 1978). Malpigmentation and incomplete eye migration are the two major malformations that routinely affect Atlantic halibut culture. Incomplete eye migration can occur in greater than 60% of an average juvenile halibut population (Harboe et al. 2009). Incomplete eye migration occurs during metamorphosis and is often associated with other malformations such as dorsal fin malformations and malpigmentation.

Malpigmentation is the most common malformation in flatfish culture (Imsland et al. 2006). The normal pigmentation pattern of Atlantic halibut is a dark (black/brown) ocular side and a white blind side. During metamorphosis, the chromoblasts differentiate into three different pigment cell types (i.e., meloanophores, xanthophores and iridophores) and the arrangement of these differentiated skin pigment cells give the halibut their specific colouring (Bolker & Hill 2000; Fujii 2000). This process is thought to be affected during development by improper nutrition, resulting in malpigmentation

(Bolker & Hill 2000). Malpigmentation can be either hyper/ hypo-melanisation, or both, resulting in albinism or ambicolouration, respectively (Chabot & Miller 2007). Albinism is the abnormal white pigmentation of the ocular side, whereas ambicolouration occurs when dark pigmentation infiltrates the white pigmentation on the abocular (blind) side. Malpigmented fish are considered inferior and will often garner a lower price when marketed whole. Although some have suggested malpigmented flatfish have improved growth performance (Heap and Thorpe 1987), others have found no difference (Imsland et al. 2006). Regardless, pigmentation is linked to a lower market price and is used as a quality criterion (Naess & Lie 1998).

The culling of malformed Atlantic halibut juveniles is a potential control strategy, but is likely to further increase the cost of juveniles. The objective of this project was to provide an epidemiological description of a commercial population of cultured Atlantic halibut and monitor their productivity over a commercial grow-out cycle. These data provide a benchmark for malformations and insight into how they impact the productivity of cultured Atlantic halibut.

1.6.2 Vaccination

Disease management information including the identification, treatment and diagnostic strategies for halibut culture is relatively sparse, particularly in the on-growing stages of production. Preventative health management measures and vaccine strategies are required to reduce the risk of infectious disease outbreaks. Prevention of disease is far preferred since economic performance is rarely optimized once treatment is necessary (Yanong 2011).

Inactivated vaccines are produced using antigens from pathogens of concern modified by various means so that they are no longer infective. The use of an adjuvant generally increases the host's response to the antigen so that immune responses are appropriately developed and primed for future exposure to the pathogen.

The properties of the ideal vaccine are:

1. Safe – for fish, the person(s) vaccinating the fish, and the consumer

2. Protective – against a broad strain or pathogen type and gives 100% protection against disease caused by pathogen or introduced by a pathogen

3. Durable – provides long-lasting protection, at least as long as the production cycle

4. Applicable – can be administered at commercial scales

5. Economical – the benefits out-weigh the cost of vaccine and its application, and any negative side-effect.

(Adapted from Yanong, 2011)

Confirming these criteria takes considerable time and expense. Atlantic halibut production has the advantage of vaccination experiences provided by Atlantic salmon production. It remains a challenge to the vaccine industry to develop inexpensive, efficient vaccines that induce lifelong protection for species that are currently produced at such small production volumes.

Several different methods of vaccine administration are used in fish, including: oral administration through feed, bath/immersion treatments and direct injection. In general, both efficacy and labour increase stepwise from oral to injection administration. Direct injection is considered the most effective technique in most situations and has the added advantage of potential incorporation of adjuvants. However, direct injection is limited because the fish must be of sufficient size to be vaccinated, and the process is labour intensive. In halibut trials, vaccination against *Vibrio anguillarum* with either i.p. injection or immersion was found to be nearly 100% effective, while anal and oral intubation provided 80% and 50% survival, respectively (Bowden et al. 2002). During early rearing, disease challenges may occur in fish that are too small or are of insufficient value to be handled and injected individually. In these situations, immersion vaccines are often employed. Adequate protection is not usually provided by oral or bath administration because antigens maybe degraded before being presented to suitable lymphoid tissues. Development of encapsulation to better stimulate gut-associated lymphoid tissues may result in better protection using this vaccine method (Bowden et al. 2002).

The combination of mineral oil based adjuvants and antigen(s) have been found effective at inducing long-lasting protective immunity, however, intraperitoneal injection of oil-adjuvanted vaccines can lead to adverse morphological and physiological side-effects in Atlantic salmon that have the potential to be as damaging as the diseases they potentially prevent (Midtlyng 1997; Midtlyng & Lillehaug 1998; Aunsmo et al. 2008). These include inflammation at the site of injection, intra-abdominal adhesions, and pigmentation (Midtlyng 1997). These physiological problems have been shown to

influence overall growth in a positive, negative, or neutral manner depending on the species studied and the combination of antigen(s) and adjuvant used. For this reason, it is important to conduct RCTs to monitor vaccine side-effects in addition to monitoring efficacy (Aunsmo et al. 2008).

1.6.3 Fish Identification

Unique fish identification is an advantage when designing commercial aquaculture trials. It enables randomized fish-level studies that can generate large datasets with adequate replication to utilize robust longitudinal statistical methods. Passive integrated transponder (PIT) tags are a proven method to individually identify fish while having low biological impacts (Navarro et al. 2006) and the ability to electronically collect tag information using scanners linked to field computers. Having limited biological effect on research subjects offers greater generalizability of study outcomes. However, since the tags are implanted internally, it is impossible to visually separate research subjects from untagged individuals. This requires all of the fish in a rearing unit to be tagged and handled, requiring thousands of fish to maintain similar production densities in commercial scale trials. The time and expense to remove these tags from the fish at the end of the study (i.e., when fish are harvested) is another task that requires consideration.

Unlike Atlantic salmon, halibut can be individually captured by divers. Having the ability to visually identify research subjects nested within commercial populations using an externally visible tag could permit individual fish trials to be conducted without the need to tag or handle the entire population of fish. The ability of divers to identify a

small minority of fish within study populations would permit the development of commercially generalizable trials if tagged fish were representative of untagged fish. This need was addressed in the third objective of the thesis, which was to determine the suitability of external loop tags to identify cage-cultured Atlantic halibut in commercial scale trials.

1.6.4 Transport

In modern day aquaculture, the movement of fish from one location to another at some point in the production cycle is unavoidable. Fish are exposed to a variety of stressors during the transport process, including high stocking densities, tank confinement, agitation, poor water quality, etc. A series of minor stressors during transport can accumulate to cause poor performance or mortality following transport. The aim of halibut transport protocols is to minimise stress by removing visual, auditory and tactile stimuli and thereby improve the welfare of the fish and lead to production efficiencies in the form of reduced mortality and increased growth performance after transport.

The flatfish aquaculture industry has two main disadvantages when it comes to transport, biology and scale. Halibut are naturally negatively buoyant, meaning they are most comfortable settled on a surface. This problem is quickly noted when producers attempt to transport halibut using equipment designed to transport Atlantic salmon. The second problem is the production scale of the flatfish farming industry in Atlantic Canada. The small volume of fish fails to attract the attention of manufactures and specialized service providers. For this reason, the majority of live haul transport

equipment in Atlantic Canada is optimised for round-fish (salmonids), which stratify themselves in the water column, making transport more efficient. Utilizing this equipment for Atlantic halibut leads to inefficient stocking densities during transport and elevated post-transport mortality. In order for the Atlantic halibut industry to develop further, transport equipment must be optimized to accommodate the biology of the species and to improve the post-transport productivity.

1.7 Summary of Thesis Objectives

An intensive randomized controlled trial was designed to accommodate individual fish-level randomization and follow-up in Atlantic halibut at a commercial marine farm. Productivity and health of Atlantic halibut in cage-culture conditions in the Bay of Fundy were investigated with the following objectives:

1) Evaluate the impact of developmental malformations and individual fish level characteristics on the productivity of Atlantic halibut under commercial culture conditions. (Chapter II)

2) Evaluate the side-effects of oil-adjuvant vaccines on growth performance, survivability and lesions of cultured halibut. (Chapter III)

3) Determine the suitability of externally fixed FT4 lock-on tags as method to identify halibut in commercial culture trials. (Chapter IV)

4) Evaluate an alternative method of transport as a means of improving the health and welfare of halibut transported overland to marine grow-out sites. (Chapter V)

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Chapter II: EPIDEMIOLOGY AND FINANCIAL IMPACT OF MORPHOLOGICAL ABNORMALITIES IN CAGE-CULTURED ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.)

Abstract

Morphological abnormalities cause production and marketing problems for cultured fish throughout the world, and are often thought to influence survival and growth. Studying the impacts of these malformations is challenging due to the difficulty of replicating commercial culture conditions in the laboratory. A population of 5244 commercially produced Atlantic halibut juveniles with a mean weight of 442g was individually tagged and visible malformations; incomplete eye migration, cataracts, malpigmentation, missing eyes, sidedness, short operculum, jaw deformities, and sex were recorded at the individual fish level. The fish were sampled regularly over a four year grow-out period at a marine cage-culture site in the Bay of Fundy, Canada. Female halibut grew faster than males, with male halibut reducing total farm gate value by 12.8%. Incomplete eye migration and cataracts had negative impacts on the growth and survival of affected individuals, reducing total farm gate value by 0.5% and 0.6%, respectively. This study provides a benchmark for the industry and identifies screening criteria for developmental malformations based on their productivity impact.

2.1 Introduction

Atlantic halibut (*Hippoglossus hippoglossus*) aquaculture is considered as an alternative to Atlantic salmon production under some circumstances in Atlantic Canada. The production goal is to harvest four to five kilogram fish following a three year grow-out period in marine cages. Currently production has considerable variation in growth and subsequent harvest size. The high cost of halibut juveniles (Shields 2001) and prolonged production cycle have important implications for the profitability of the industry (Ridler 1995).

Developmental malformations result in financial losses for producers of many marine finfish species. Most gross malformations result in compromised swimming and feeding ability and consequently reduce growth, population uniformity and market value (Leatherland & Woo 1999). Considerable work has been completed on diets (Saele et al. 2003) and rearing conditions (Mangor-Jensen et al. 1998) to reduce the prevalence and severity of these malformations in a variety of marine aquaculture species. In general, abnormalities cause decreased growth rates, elevated mortality and decreased final product value in terms of quality, quantity, and market appeal (Fraser & de Nys, 2005; Shields 2001). Morphological abnormalities are typically associated with increased susceptibility to disease (Paperna 1978). Malformations often lead to primary downgrading of product as malformed fish can be difficult to process or simply fail to meet consumer expectations which are formed from experiences with wild fish which are rarely observed with malformations. It is important that reasons for primary downgrading are identified so that problems can be prioritized and addressed based on

greatest economic concern (Michie 2001). This is particularly important in alternative aquaculture species where economic viability can be tenuous.

Abnormalities are common and can be severe in marine fish species due to complicated early rearing requirements (Planas & Cunha 1999; Shields 2001). The factors leading to developmental malformations in flatfish culture are complex and not completely understood (Pittmann et al. 1998). Causes are known to include diet (Hamre et al. 2005; Shields 2001) feeding rates and schedules, photoperiod (Harboe et al. 2009), tank colour (Yamanome et al. 2005) and other subtle environmental factors (Pittmann et al. 1998). Adequately managing these factors during commercial production can be technically challenging (Shields 2001).

To date, little has been published on the prevalence of abnormalities in culture operations and their influence on production, particularly in cage-culture growing operations. The majority of studies on developmental abnormalities in fish are largely descriptive with only a few abnormalities explored beyond the level of association with particular causal factors (Leatherland & Woo 1999).

Atlantic halibut undergo a complicated metamorphosis from a bilaterally symmetric pelagic larva to an asymmetric benthic juvenile (Saele et al. 2003). It is necessary that this delicate developmental period is adequately accommodated by the conditions of commercial hatchery production.

The type, prevalence, and severity of malformations are highly variable between batches of halibut in commercial culture (Pittmann et al. 1998). A seemingly small or even unnoticed aberration to husbandry protocols can result in high levels of

developmental abnormalities (Pittmann et al. 1998). Recent advances in intensive hatchery methods have greatly reduced the occurrence of malformations in Atlantic halibut juveniles (Brown 2010; Harboe et al. 2009) . However, malpigmentation (Bolker & Hill, 1999; Imsland et al. 2006), incomplete eye migration (Saele et al. 2006), anterior dorsal fin abnormalities (Pittman et al. 1998) and cataracts (gross opacity of the lens) (Treasurer et al. 2007) remain common sub-lethal abnormalities in farmed Atlantic halibut along with various other forms of eye damage (Remø et al. 2011). Due to the anatomical prominence of both eyes located dorsally on their head, missing (Greaves & Tuene 2001) and damaged eyes (Williams & Brancker 2006) are common in farmed halibut.

This study provides an epidemiological description of fish characteristics in a commercially-reared population of Atlantic halibut. An observational cohort study was used to observe the impact of these characteristics on the growth and survival of cultured Atlantic halibut at levels important to commercial growers. This information is important for growers to make evidence-based decisions regarding the quality of juveniles purchased and their potential impact on profitability.

2.2 Material and methods

2.2.1 Study population

On 14 March 2006, a population of 5244 Atlantic halibut juveniles held at a commercial fish hatchery were anesthetised and tagged intraperitoneally with Passive Integrated Transponder (PIT) tags (AVID Technology, Tewksbury, MA, USA). Tags were inserted into the peritoneal cavity (Fig. 2.1) through a small incision made by the

partial puncture of a 12-gauge hypodermic needle on the blind side of the fish, similar to the process described by Gries & Letcher, (2002) for Atlantic salmon. Anaesthetic (Tricaine methanesulfanate (TMS), Syndel Laboratories Ltd., Qualicum Beach, B.C, CAN) baths were used at all handling points during the study at a concentration of 150 mg/l.

The trial began 3 May 2006 (day 0) when all fish were weighed (mean weight=442g, SD=128g) and assessed dichotomously (presence/absence) of individual unique characteristics. At this time, fish were removed from two tanks (Pre), randomized to treatment groups for a separate study (Chapter III) and then systematically assigned to one of two tanks (Post) where they were held until transport to the marine growout site on either July 3 or July 5, 2006 (representing day 63 and 65, respectively).

2.2.1.1 Land-based marine early rearing site

The PIT tagged population was held in two separate tanks at a recirculating land-based facility under normal husbandry conditions to allow their incisions to heal. All fish were part of a larger vaccination trial (Chapter III). Vaccination status was controlled analytically in this study to remove any potential confounding effect of vaccine. Water temperature at the land-based facility ranged from 8.0 to 13.3°C.

2.2.1.2 Marine grow-out site

The field monitoring of growth and survival took place at a commercial halibut farm in Lime Kiln Bay, part of the Bay of Fundy in New Brunswick, Canada (Latitude: 66˚49’50.66”N Longitude: 66˚49’50.68”W) (Appendix 1). Lime Kiln Bay is a small

protected bay, hosting several Atlantic salmon aquaculture sites and a single site culturing mussels (*Mytilus edulis*) operating in close proximity. The temperature ranged from 0.5°C - 14.5°C during the study period. Fish were fed daily to satiation with a commercial diet (AquaSea ™, Corey Feed Mills Ltd., Fredericton, New Brunswick Canada). The pellet size was increased from 10mm to 13mm in May 2007 to reflect the increased size of the fish. The fish were housed in a single 70 m circumference Polar Circle sea cage fitted with treated nylon net sides and a flat tensioned Dyneema® (DSM Dyneema, Stanley, North Carolina, USA) bottom net.

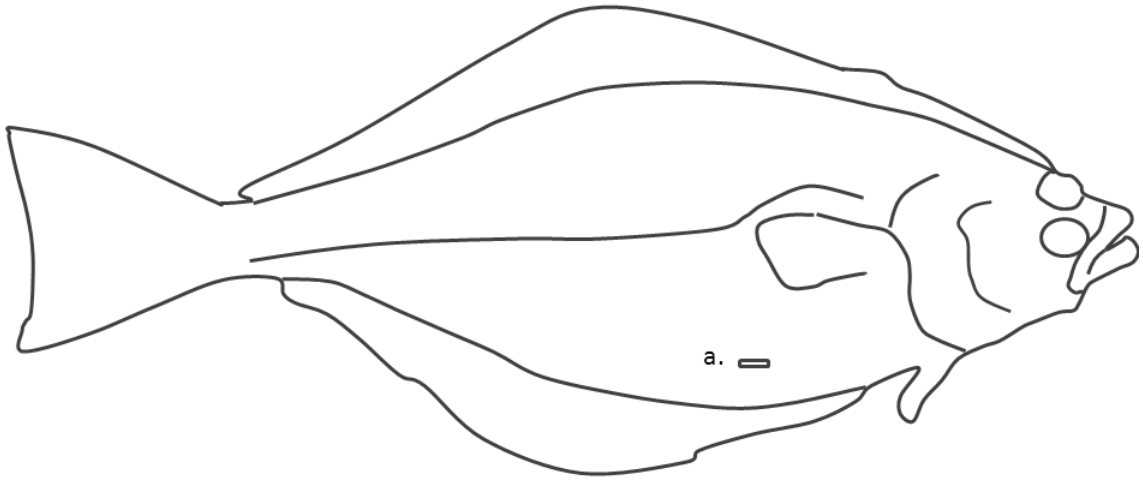

Fig. 2.1. Passive integrated transponder (PIT) tag location (a.), inserted into the peritoneal cavity from the blind side of the fish.

2.2.2 Field sampling and monitoring

Fish were sampled on days 0, 189, 372, 554, 769 and 1105 of the study and at harvest, which ranged from days 1294 to 1539. At each sampling, the majority of the population was crowded using a seine net, removed using a dip net, and placed in an anaesthetic bath. Each fish was then measured (weight and length), and observed for external health characteristics. Incomplete eye migration was scored dichotomously at the beginning of the trial. As the trial progressed it became evident that incomplete eye migration was an important predictor of productivity. To establish if there was a dose-response relationship between the severities of incomplete eye migration, all remaining fish were re-evaluated using a four-point incomplete eye migration score (Fig 2.2) on day 372 of the study.

Prior to initiation of the trial, fish which were identified as missing both eyes were excluded from the study. Fish missing one eye (i.e., complete removal of the globe or damage/malformation to the point of non-function) were included in the study and recorded.

Malpigmentation was classified by visual assessment as occurring when greater than 25% of the ocular side was white, a level which was judged to be excessive and abnormal for properly developed halibut juveniles. If any gill filaments where exposed when the operculum was closed, the record reflected “short operculum” regardless of severity. All others were recorded as “normal operculum”. Cataracts, defined as a loss of transparency in the normally clear lens of the eye (Treasurer et al. 2007), were assessed

visually without distinction between unilateral and bilateral cataracts. Sex was identified and recorded at necropsy when collected as a mortality or at harvest.

Mortalities were identified and necropsied as they were recovered by divers on a weekly basis. During a planned sampling on day 189 of the study, a technical problem with a seine net resulted in the mortality of 51% (2712) of the study population. Approximately four hours after crowding was initiated, signs of distress were observed and the procedure was immediately discontinued. All mortalities were collected and necropsied the following day.

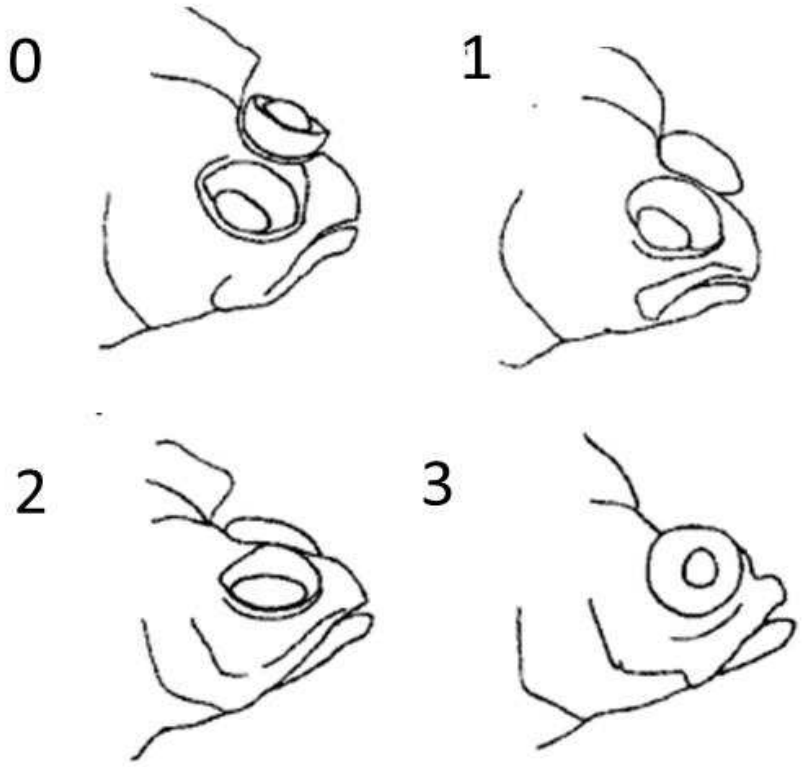

Fig. 2.2. Categories used to classify incomplete eye migration status in halibut juveniles: (0) complete migration with both eyes located on the ocular side with the lenses of both eyes visible. (1) Full diameter of migrating eye visible, but lens not visible. (2) Migrating eye just visible. (3) Blind side eye not visible. All fish were placed on a flat surface and viewed from directly above during classification (Adapted from Gara et al 1998 and used with permission - Appendix 2).

2.2.3 Statistical analysis

All data were analysed using the statistical package STATA 11.0 (STATA, College Station, Texas, USA). A probability level of $P<0.05$ was considered significant for all tests.

2.2.3.1 Descriptive statistics

The prevalence of abnormalities observed in the population is presented in Table 2.1. A Chi-square statistic was used to explore potential associations and co-morbidity of recorded abnormalities. The severity of incomplete eye migration was reassessed using a four point scale on day 372 of the study. However, approximately 50% of the study population had previously exited the study and thus were unavailable for reassessment. Limiting the analysis to fish that were available for reassessment restricts the interpretation of the data to fish that survived the stressful handling event, which has potential to bias the results. For this reason a number of analytical options where considered (Appendix 3.). For brevity the best available option is reported. Fish that were unavailable for reassessment where assigned a score for incomplete eye migration based on their original (dichotomous) incomplete eye migration score and a weighted average of the distribution of incomplete eye migration severity in the remaining (reassessed) population. This approach provides an unbiased, population-averaged estimate of incomplete eye migration on productivity.

2.2.3.2 Data handling, checking and restriction

Outlying measurements that were obvious typographical errors (i.e., measurement in question is inconsistent with proceeding and following measurements) were removed and treated as 'missing'. Otherwise all outlying observations were retained in the analyses. Malformations with prevalence less than 0.5% were excluded from model building.

2.2.3.3 Unconditional associations

All variables (Table 2.1) were examined for unconditional associations with the outcome weight (W) using a mixed multilevel model with an unstructured zero banded correlation matrix. All predictors with a *P*-value less than the liberal cut point of 0.20 were retained for model building.

2.2.3.4 Multivariable methods

Measurements of W were statistically analysed using a multilevel mixed model that included within-fish covariance structures. The weight data comprised a series of six time points with outcome variance increasing over time. An unstructured correlation matrix was chosen due to varying length of time between samplings with seasons alternating (winter & spring / summer & fall) between the samplings.

Models were fit with maximum likelihood estimation to appropriately evaluate the fixed effects of the model. The W data were natural log transformed (ln) so that model residuals followed the assumption of normality with the natural log of initial weight (W_0) was included as a covariate. The model was built using stepwise selection.

All biologically important two-way interactions between all predictors were included in model building and were assessed for significance (determined by a likelihood ratio test). Interactions were retained in the model when they improved overall model fit as determined by the Akaike Information Criterion (AIC: Dohoo et al. 2009a).

Large variation in productivity attributed to seasons was observed between the periods. The variable 'period' was forced into each model, and its interaction with all predictors was explored. The inconsistent duration of periods and relatively long follow-up period suggested a non-stationary covariance would best explain the correlation of fish level observations over time while controlling for the increasing variance observed in the outcome over time. The appropriate number of bands was assessed by incrementally increasing the number of bands until the optimal model fit was reached as indicated by the AIC.

2.2.3.5 Survival analysis modeling

The impact of individual morphological abnormalities on individual fish survival was analysed using a Cox proportional hazards model. Time to mortality was the outcome. Dichotomous predictors were screened for significant associations with mortality using a log-rank test and a liberal P-value of 0.20. The continuous predictor initial weight was assessed for linearity with the outcome by examining martingale residuals plotted against initial weigh. A square root transformation provided a linear relationship. The hazard attributed to abnormalities was found to vary with time. The data were split at each exit point so that interactions with time could be considered to correct for non-proportional hazards. The proportional hazard function was observed

graphically to evaluate proportionality and tested using a global test (Dohoo et al. 2009b). In order to satisfy the proportional hazards assumption, it was necessary to rescale (ln) the time variables used in interactions.

2.2.3.6 Economic valuation of unique characteristics

Farm financial losses due to observed morphological abnormalities were estimated using the measured losses in productivity. The difference between the average harvest weight of an ideal fish, completely free from malformations, and the average weight of a fish with a single morphological deformity was then scaled to production volumes of a small commercial farm of 96,000 halibut (Table 2.2). Prices were set at $12.10/kg for head on gutted fish based on current industry prices and evisceration losses at processing were assumed to be 10% for males and 5% for females, based on field observations. The prevalence of malformations used for the economic valuation reflected those observed in the study.

Table 2.1. Descriptive statistics of individual fish characteristics and potential study confounders (n=5244)

Variable	Status	Prevalence (%)	95% CI
Sex (male or female)	Male	49.8	48.3-51.3
Cataract (present/absent)	Present	5.3	4.7-5.9
Incomplete Eye Migration (4 categories)	None	65.9	63.9-68.0
	Mild	17.2	15.6-18.9
	Moderate	9.4	8.1-10.6
	Severe	7.5	6.4-8.6
Sidedness -Sinistral Eye (yes/no)	Yes	8.2	7.4-8.9
Malpigmentation (present/absent)	Present	6	5.5-6.8
Short Operculum (yes/no)	Yes	5.6	5.0-6.2
Eye Missing	One	10.9	10.6-11.3
	Both	0.03	0.03-0.04
Tank Number Pre. (one or two)	One	43.6	
Tank Number Post. (one or two)	One	49.3	
Jaw Deformity (present/absent)	Present	0.1	0-0.2

2.3 Results

2.3.1 Prevalence and co-morbidity

Halibut with incomplete eye migration were twice as likely (OR = 1.96, 95% CI 1.52-2.53) to have one or more cataracts than halibut with normally migrated eyes (χ^2= 39.6, $P < 0.01$). Females were also less likely to have a cataract(s) than males (OR=0.68, 95% CI 0.52, 0.90) (χ^2= 8.05, $P < 0.01$). Left-sided halibut were less likely to have short operculum (OR =0.34, 95% CI 0.15 - 0.67). No other statistically significant correlations were observed between morphological abnormalities.

2.3.2. Growth analysis

A complete list of morphologic abnormalities and husbandry factors are presented in Table 2.1. Eleven different variables were available to build models (including W_0). Two husbandry variables, tank. at hatchery pre-vaccination (tank number pre.) and tank at hatchery post-vaccination (tank number post.) appeared to be associated with growth (P<0.20). However, because the prevalence of malformations was different in the two pre-vaccination tanks this variable was considered an intervening variable and excluded from further analysis. The eight remaining variables were retained for multivariable analysis (Table 2.3). Jaw deformity and shortened operculum were removed during the unconditional association's analysis stage.

Sex was the single largest factor affecting overall growth (Table 2.3), with females performing significantly better than males (P<0.001) for every assessment point (Fig 2.3).

Halibut with moderate and severe forms of incomplete eye migration weighed significantly less than fish with normal migration over the course of the study (Fig 2.4).

Table 2.2. Production assumptions of a small scale commercial Atlantic halibut (*Hippoglossus hippoglossus*) cage-culture farm used in the economic analysis.

Variable	Value
Price of halibut ($/kg)	12.1
Dress out (%) - Female	5
Dress out (%) - Male	10
Stocking per cage (n)	8000
Cages per farm	12
Total fish (n)	96000
Time to harvest (months)	45

Table 2.3. Impact of unique characteristics and malformations on productivity and farm economics. All calculations are prior to downgrading and without considering losses to mortality and assume an annual production of 96,000 halibut.

Variable	Mean Harvest [1] Weight (g)	95% CI	Weight [2] Reduction (g)	Prevalence (%)	Fish[3] Affected (n)	Cost ($)[4]	% farm[5] gate value
Ideal	3149.2	(3077.5-3222.6)	-	-	-	3,658,123	-
Tank Post-vaccination	2319.9	(2286.0-2354.2)	78.3	49.3	47328	44,838	1.2
Male	2009.9	(1972.6-2047.8)	768.01	49.8	47808	466,493	12.8
Eye(s) missing	2319.3	(2270.7-2368.9)	39.04	11.3	85114	5,143	0.1
Cataract	2006.4	(1947.2-2067.5)	364.36	5.3	5088	22,432	0.6
Migration Mild	2275.4	(2233.6-2317.9)	-46.15	17.2	16512	-9,220	-0.3
Migration Moderate	2143.1	(2100.4-2186.5)	86.21	9.4	9024	9,414	0.3
Migration Severe	2127.1	(2083.9-2171.2)	102.06	7.5	7200	8,891	0.2

[1] Fish weight at end of grow-out period

[2]The reduction in harvest weight from ideal fish to fish with specific abnormality as predicted by the multi-level model

[3] Number of fish out of 96,000 that have the condition

[4] The financial cost of individual abnormalities assuming a market price of $12.10/kg

[5]Amount of farm gate value lost for each abnormality using a population of 96,000 “ideal” fish as a reference population

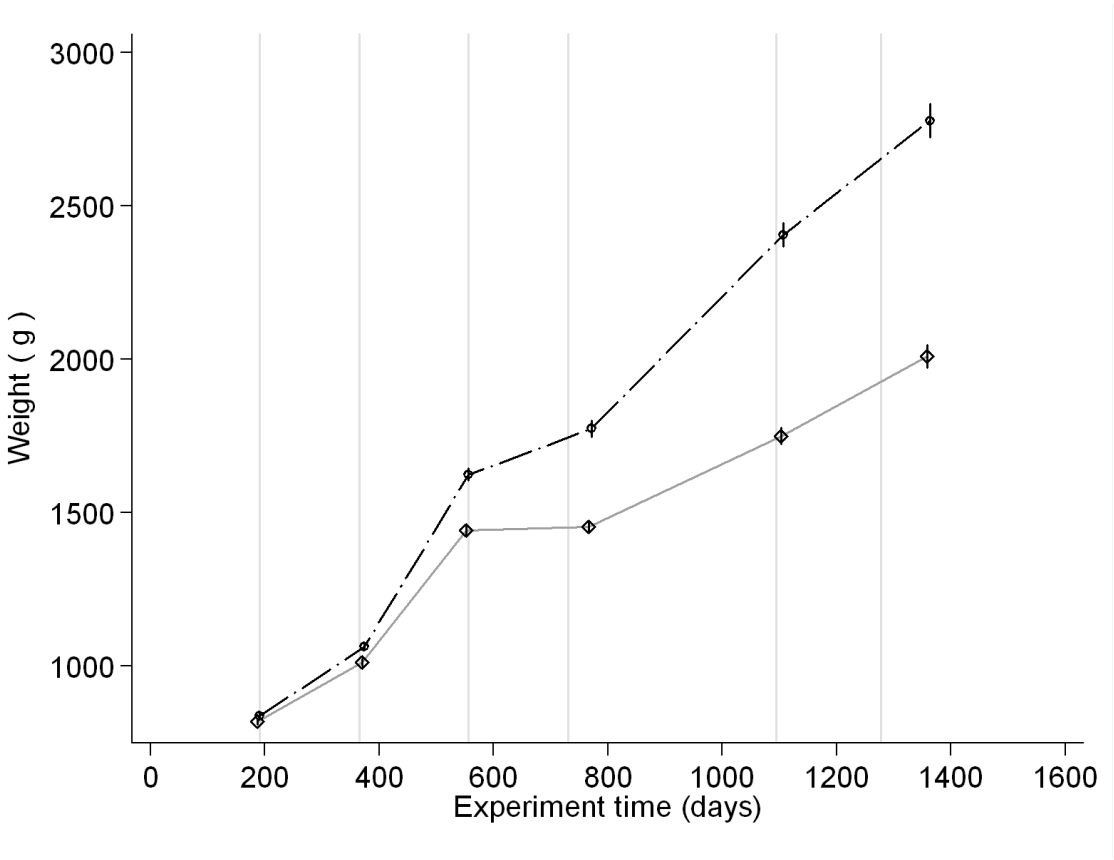

Fig. 2.3. Mean weight profile with 95% CI of male and female Atlantic halibut over a cage-culture growout.

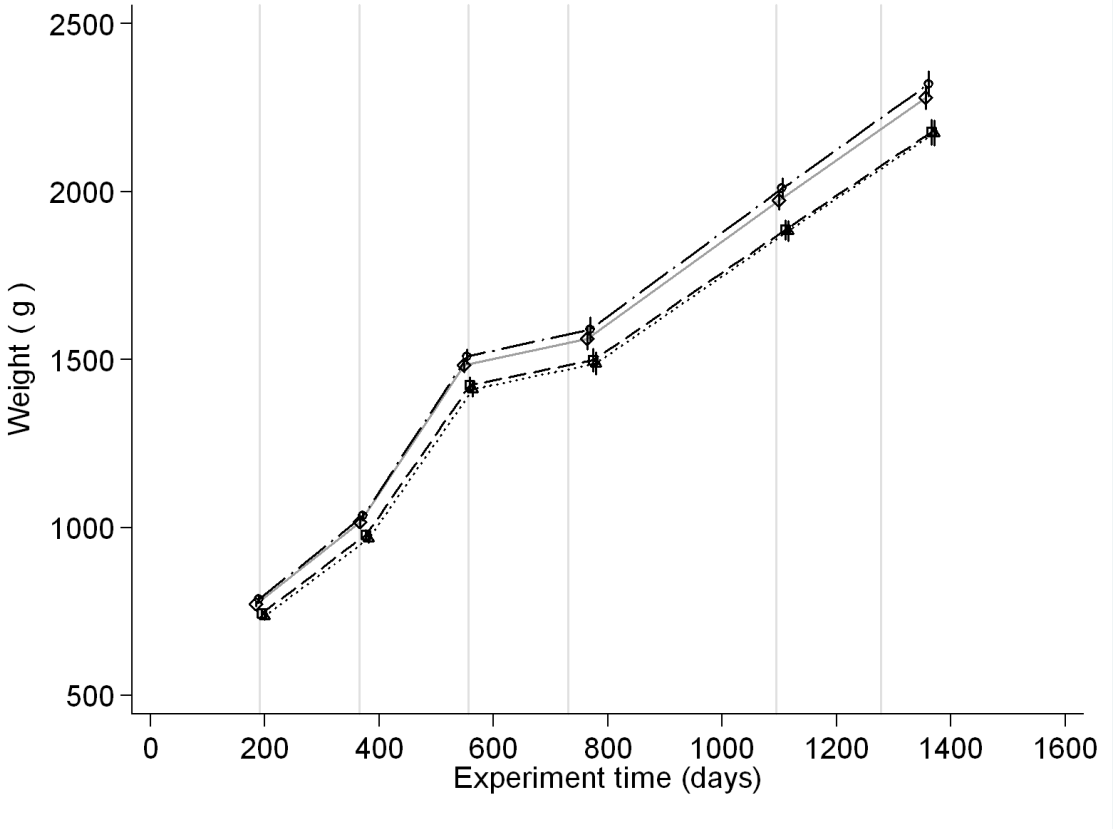

Fig. 2.4. Mean weight profile and 95% CI of normally migrated (solid) Atlantic halibut and those with mild (dash dot), moderate (dash) and severe (dot) cases of incomplete eye migration.

Fish with mild incomplete eye migration weighed significantly more than fish with normal eye migration (Fig 2.4).

2.3.3 Survival analysis

In the early points of the production cycle cataracts and incomplete eye migration were not significantly associated with mortality, but with time, their hazard of mortality increased and became significant on days 100 (Fig 2.5a) and 125 (Fig 2.5b), respectively. The hazard ratios from the final Cox proportional hazard model for the midpoint of the study are presented in Table 2.4 with the range of hazard values from day 0 of the study to day 1105. The hazard functions for each significant predictor (cataracts, incomplete eye migration, and initial weight (W_0) were changed over the progression of the study (Fig 2.5).

On average, cataracts significantly increased the mortality hazard, with a hazard ratio of 3.27 (95% CI: 2.12-5.07) at the mid-point of trial. The hazard ratio varied from 0.29 at the beginning of the trial to 4.28 at the end of the trial, indicating that cataracts increased the hazard of mortality in later periods more so than in earlier periods (Fig 2.5a)

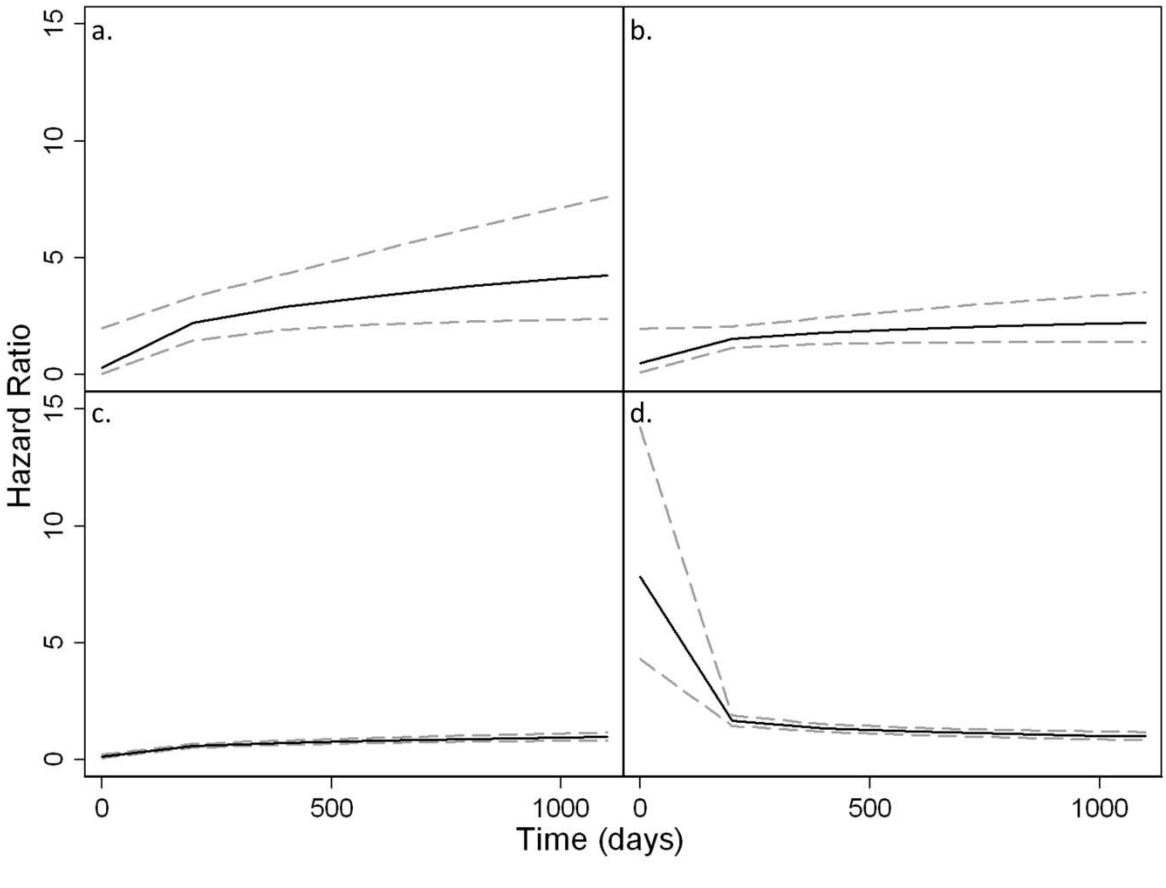

Fig. 2.5. Hazard ratio profiles and 95% CI (dashed lines) from a Cox proportional hazards model with time as the covariate. Plotted are the individual significant predictors cataracts (a.), incomplete eye migration (b.), above average initial weight (c.), below average initial weight (d.) on the hazard of mortality over the study.

Table 2.4. Cox proportional hazards model results for abnormalities in Atlantic halibut juveniles over the 1105 day study period. A population of 5244 Atlantic halibut were followed with 254 mortalities recorded.

Variable	Categories	HR[1]	95% CI[1]	Range[2]	*P* -value
Cataract	0 Absent	-	-	-	-
	1 Present	3.27	(2.12, 5.07)	(0.29- 4.28)	<0.001
Eye Migration	0 Absent	-	-	-	-
	1 Present	1.93	(1.38, 2.71)	(0.49 - 2.24)	<0.001
Initial weight	100g increase	0.81	(0.71, 0.92)	(0.13 - 0.99)	<0.001

HR - Hazard Ratio

[1-] At midpoint of the study (day 554)

[2] HR at day 0 and day 1105

2.4 Discussion

The magnitude of unexplained variation in growth models suggests that other unquantified factors are important in predicting the growth performance of juvenile Atlantic halibut. Abnormal halibut with reduced growth were similarly sized at the beginning of the trial because the fish were graded into similar size categories prior to shipment. Thus, only small differences in average weight were observed between fish with differing characteristics at the beginning of the study.

Considerable size variability among aquaculture stock is largely explained by differential growth rates resulting from inherent genetic differences in the growth capacity of the fish (Sunde et al. 1998*)* and not in factors that are measurable in commercial culture. Current juvenile production is primarily based on wild caught and F1 broodstock (Jackson et al. 2003) which leads to highly variable growth rates as compared to more domesticated fish stocks (i.e., cultured Atlantic salmon; Dahle et al. 2006).

Randomization enables the allocation of these unmeasured variables in unbiased and comparable proportions to the treatment groups being studied. In the cage-culture environment, sex as well as moderate and severe forms of incomplete eye migration, were found to have significant and consequential impact on growth. A strong tank effect in which the variation of a tank group is large relative to among fish variation within a tank group was observed in the hatchery, confirming the importance of rigorous randomization to control for factors such as “tank effect” in aquaculture studies (Speare et al. 1995).

The effect of vaccination status was balanced across other factors by randomizing the treatment groups in addition to being analytically controlled. Although the derived model describes the occurrence of abnormalities and their influence on growth, there are many other potential factors that could not be recorded or analysed, such as concurrent disease, genetic background and hierarchical status.

2.4.1 Sex

Male fish were found to have a significantly reduced size at harvest compared with females. The growth of male and female fish was comparable until the third measurement period (day 554) immediately after the second sea winter. The majority of weight gain for maturing male fish during this period was gonadal growth which was released as milt or reabsorbed, resulting in poor somatic growth. Females mature at a much larger size (McCracken 1958) and are harvested well before maturation so the loss of somatic growth to maturation is not apparent. The high impact of gender on overall growth in combination with the relatively equal ratio of male to female fish, make sex the single most important characteristic impacting overall farm productivity. The use of all-female populations has been targeted to improve overall farm productivity

2.4.2 Cataracts

The eyes of halibut are particularly vulnerable to developing keratitis and cataracts given their exposed placement on the head. It is not surprising that fish with incomplete eye migration are more likely to have cataracts as they are likely to contact the substrate resulting in physical trauma (Treasurer et al. 2007; Remø et al. 2011). Significant reduction in harvest weights of fish with cataracts provides further evidence

of this detrimental factor. Eye deformities such as cataracts have the potential to be lethal (Noble et al. 2011) because damage to the eyes can affect behaviour and cause physiological stress (Thatcher 1979). Cataracts causing visual impairment may reduce feeding ability and reduce aggression avoidance behaviour directed by other halibut (Noble et al. 2011). The increasing hazard of mortality observed for halibut with cataracts suggests that the condition worsens over time or that cataracts are components of an overall degradation in health.

2.4.3 Incomplete eye migration

The observation that halibut with incomplete eye migration had reduced growth was expected. The relatively high prevalence of this abnormality and its significant impact on growth impacted total harvested weight and farm gate value. Additionally, incomplete eye migration was an important predictor of survival. The early observations of the importance of incomplete eye migration led to a later change in classifications, so that severity of the condition and its influence could be assessed. A large number of halibut died prior to the re-assessment of incomplete eye migration severity, because of this a large number of observations from early measurements were excluded when this variable was used in subsequent models. To avoid this loss of data, eye migration severity scores of fish unavailable for reassessment were estimated using the *a priori* dichotomous data, which were modelled into a categorical variable with three levels (mild, moderate and severe) using a randomized procedure.

Although this method was not a true measure, the procedure was unbiased and permitted more complete use of the data. This allowed us to determine if mild forms of

incomplete eye migration are similar to normally migrated halibut from a growth stand point and therefore, when grading juvenile stock producers, should limit culling to fish with moderate and severe forms of incomplete migration only. This assumes halibut with mild incomplete eye migration do not pose a marketing problem for producers, and force downgrading.

2.4.4 Sidedness

Flatfish species are predominantly right sided (dextral) or left sided (sinistral) with Atlantic halibut classified as a right eyed flounder. On occasion some halibut may be sinistral but completely normal in all other respects (Diaz De Astarloa 1997) this is known as reversal. The prevalence of eye reversal in other flatfish species has been documented as low as 4.4% in laboratory reared summer flounder (*Paralichthys dentatus*) (Diaz De Astarloa 1997) and up to 40% in California halibut (*Paralichthys californicus*) (Kramer et al. 1995). Reversal occurred in 8.2% of the study populations. Although sinstral halibut had no apparent decrease in growth or survival and would likely go unnoticed by the average consumer, it does have the potential to create difficulties for automated processing and vaccine administration resulting in inefficiencies for commercial operations.

2.4.5 Pigmentation abnormalities

Malpigmentation is one of the most prevalent morphological abnormalities in flatfish culture (Imsland et al. 2006). Two separate pigmentation concerns arise in flatfish: black pigmentation (melanisation) (Bolker & Hill 2000) on the normally white abocular side, referred to as ambicolouration or staining. The second is a form of

albinism that results in a hypopigmentation or a lack of pigmentation (i.e., beyond normal white markings) on the normally black coloured ocular side. Malpigmentation was not found to influence growth and survival over the course of the grow-out period. Consistent with our findings, other studies have found that hypopigmentation of the ocular side has no impact on growth (Imsland et al. 2006). However, these pigmentation patterns are distinctly different from those of wild fish and so can affect the market value of cultured fish (Yamanome et al. 2005).

2.4.6 Farm management considerations

The high initial cost of halibut juveniles results in high stocking and financing costs, increasing the financial risk for halibut farmers. This reality makes it economically unfavourable to raise poor performing fish as it will lengthen the grow-out period. When purchasing stock, farmers should assess juveniles for quality and potential growth and survival performance based on criteria with supporting evidence of their effect on productivity. The prevalence of fish with malformations not only limits production but can have important health management and husbandry considerations. Differing growth performance increases size variability within a population, resulting in fish reaching harvestable size over an extended period. This requires additional grading and handling or acceptance of undersized fish at harvest time. Culling poorly performing stock must be balanced against further grow-out time that underutilizes farm resources and causes delays to farm fallowing and restocking plans (i.e., practicing all-in all-out farm management). This provides farmers with economically sound justification for discriminating against malformed stock or negotiating a reduced price to offset the negative effect on productivity.

The farm gate losses reported are simply the direct costs associated of reduced growth and do not include costs that arise from downgrading if the final product fails to meet the customers' expectations. These losses are also distinct from those that result from increased risk of mortality that malformations (e.g. incomplete eye migration or cataracts) may cause. Overall, mortality losses were minor and it would be difficult to determine to what level malformation increases this cost because losses are time sensitive, whereby mortality occurring later in the production cycle results in greater losses due to the increased opportunity costs and feed costs.

In conclusion, estimates of the prevalence and severity of malformations (e.g. incomplete eye migration and cataracts) are useful predictors of productivity prior to purchase. Having an understanding of the prevalence and the impact of these malformations allows farmers to make more informed management decisions and be better informed on the financial burden of these malformations.

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Chapter III: THE IMPACT OF OIL-ADJUVANT VACCINES ON THE PRODUCTIVITY OF COMMERCIALLY CULTURED ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.)

Abstract

Atlantic halibut were randomly allocated to one of three oil-adjuvant vaccines or one of two control treatments and were monitored over a commercial grow-out cycle in a single cage at a marine site. In addition one vaccine was injected in two different locations to determine an optimal injection location. Survival and growth were monitored throughout the grow-out cycle, and assessments for intraperitoneal (i.p.) adhesions were performed on mortalities and at harvest. Non-injected and saline injected controls had significantly higher growth than two of the oil-adjuvant vaccine groups in the first 189-day growth period ($\chi^2_{(6)}$=104.39 P<0.01) following vaccination. Growth was not significantly different between vaccinated and control fish in all later time periods, and no significant differences in weight were observed at final harvest. Although vaccination did not significantly influence survival during the trial, protection was not quantifiable due to the lack of a natural pathogen challenge. Vaccinated halibut had a significantly higher prevalence and greater severity of i.p. adhesions as compared to controls. In conclusion, oil-adjuvant vaccines did not have negative side-effects that would prevent their use in future vaccine development in Atlantic halibut.

3.1 Introduction

Disease is the single largest constraint and cause of economic loss in commercial aquaculture production (Subasinghe et al. 2001; Francis-Floyd 2005; Subasinghe 2009). With the increased economic importance of aquaculture worldwide, vaccines for several bacterial fish diseases have been developed and vaccination has become central to preventing infectious disease in commercial finfish production (Lillehaug et al.1992; Haskell et al. 2004). Furunculosis and vibriosis are examples of two serious and economically important diseases that for the most part are well controlled by vaccination in the production of Atlantic salmon (*Salmo salar*) globally (Lillehaug et al. 2003). Vaccination protocols in combination with good management practices have contributed to dramatically reducing mortality and antibiotic use despite substantial increases in production (Lillehaug et al. 2003; Grave et al. 2008). In recent years, however, there has been increased antimicrobial use in aquaculture in Norway due to increased production of alternative aquaculture species like Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*) (Grave et al. 2008).

Despite the overall benefits of vaccination, it is not without compromise and can be a difficult decision in some cases (Thorarinsson & Powell 2006). Producers must weigh the known costs of vaccination against the generally unquantified risk of disease. The direct costs of vaccination include the cost of the vaccine and its administration, and although the cost per-dose is relatively small, it must be considered in the context of commercial scales. The indirect costs of vaccination are often more subjective and unpredictable. Vaccine side-effects are one of the largest indirect costs of vaccination. These side-effects often present as intraperitoneal adhesions, melanisation of serosal

surfaces, and reduced growth. The health and welfare of the fish, as well as their market value at harvest, are often affected by these negative side-effects (Midtlyng 1997; Midtlyng & Lillehaug 1998; Sørum & Damsgård 2004). Unavoidable indirect side-effects often include a period of reduced appetite and growth following vaccination related to the stress of anaesthesia, handling and vaccination (Midtlyng 1997). The majority of vaccine efficacy and side-effect testing has been studied in Atlantic salmon (*Salmo salar* L.) under laboratory settings. Although laboratory assessments offer speed and efficiency while maintaining control of extraneous factors extrapolation of their results to commercial culture conditions is challenging (Aunsmo et al. 2008; Burnley et al. 2010). Production factors may interact with treatments to mask any benefits of the vaccine. Laboratory trials run the risk that they may fail to reach scales where production side-effects can be noted (Aunsmo et al. 2008; Mitchell 1997).

Mineral oil is the most commonly used adjuvant in fish vaccines because it permits a slow release of antigen in the body lumen. This allows for a prolonged contact between the antigen and the immune system while stimulating the non-specific immune system and increasing the overall immune response (Ellis 1988). These advantages come with some drawbacks; oil-adjuvant vaccines elicit intraperitoneal lesions in the form of adhesions and melanin deposits on serosal surfaces and have been previously documented in Atlantic salmon (Ingilae et al. 2000; Midlyng et al. 1996; Midtlyng 1997; Lillehaug et al. 1992), Arctic charr (*Salvelinus alpinus*) (Pylkko et al. 2000), Atlantic cod (Hamid 2003; Mikkelsen et al, 2004) and Atlantic halibut (Bowden et al. 2003). Adhesions can restrict the normal movement and function of internal organs and potentially restrict the esophagus, preventing the passage of food to the stomach (Lars

Speilberg per.comm). Side-effects are often associated with reduced growth and quality related downgrading at harvest (Midlyng et al. 1996; Midtlyng 1997; Lillehaug et al. 1992; Michie 2001). For this reason, there has been continued work to find adjuvant formulations that increase immune response to antigens but with minimal or no side-effects (Midtlyng et al. 1996).

Although vaccines are not a substitute for good management and biosecurity (Haskell et al. 2004), any protection afforded by their use can help protect aquaculture stocks when exposure to infectious disease is unavoidable. Provided they do not induce harm, utilizing salmon vaccines for alternative species such as Atlantic halibut or Atlantic cod generates advances in vaccine availability prior to industry production volumes warranting the investment by commercial health service companies into species-specific vaccines. However, assessments of their true value and cost require evidence building regarding the benefits and potential disadvantages.

Species anatomy and physiology may contribute to differences in the side-effects of vaccines, which require further evaluation before employing widely (Pylkko et al. 2000). Atlantic halibut develop an adequate immune response with few physical side-effects when vaccinated with mineral-oil based adjuvant vaccines (Bowden et al. 2003). However, there have been no studies detailing the impact these vaccines have on growth or survival when halibut are raised in commercial situations. The experience of the industry with commercial-scale halibut production and related disease is limited but increasing as farming practices expand globally. Currently, no vaccines are registered for Atlantic halibut in Canada, leaving the industry at a possible disadvantage when managing infectious disease in this species. Optimizing the injection methods for flatfish

and identifying the optimal timing for vaccination (size/age) are also factors that require further study (Gudmundsdottir et al. 2003).

The objective of this controlled trial was to monitor the productivity of a cage-cultured population of Atlantic halibut vaccinated treated with different vaccines over a grow-out period in Atlantic Canada.

3.2 Materials and methods

3.2.1 Study population

Atlantic halibut (n=5244) held at a commercial fish hatchery, were individually anaesthetised using tricaine methanesulfanate (TMS), (Syndel Laboratories Ltd., Qualicum Beach, B.C, CAN,www.syndel.com) at a dose of 150 mg/l and tagged intraperitoneally (i.p.) with PIT tags (Avid Identification Systems Inc. Norco CA, USA, www.AvidID.com). PIT tags were inserted into the peritoneal cavity through a small incision made by the partial insertion of a 12-gauge hypodermic needle on the blind side of the fish, similar to the process described by Gries & Letcher (2002) for Atlantic salmon. Fish were maintained at the hatchery for 50 days to allow the incisions to heal prior to vaccination. A complete timeline of all major events for the study population is summarized in Table 3.1.

Table 3.1 Time table of events leading up to and throughout the study.

Study event	Completion date	Days since vaccination
Passive inegrated transponder tagging	14 March 2006	-50
Pre vaccination health screening	15 March 2006	-49
Vaccination	3 May 2006	0
Transfer	July 3-4 2006	63-65
Health and Productivity Sampling 1	8 November 2006	189
Fall Mortality Event	9 November 2006	190
Health and productivity sampling 2	10 May 2007	372
Health and productivity sampling 3	8 November 2007	554
Health and productivity sampling 4	11 June 2008	770
Health and productivity sampling 5	12 May 2009	1105
Ongoing mortality sampling	3 May 2006 - 19 July 2010	n.a.
Harvests	17 November 2009 - 19 July 2010	1294-1538

n.a. = not applicable

3.2.2 Vaccine treatments

The trial began 3 May 2006 (day 0), at which time fish had a mean weight of 442 g (SD= 128). Following crowding, the fish were removed from the rearing tanks using a dip net and placed in anesthetic baths (all anesthetic baths used for the duration of the trial were TMS at 150mg/l). PIT tags were scanned to identify a pre-assigned random allocation to one of seven treatment groups. Two controls were used; a saline-injected group to compare the impact of the vaccines and, a non-injected control group to evaluate the injection process. Three different oil-adjuvant salmon vaccines were tested (Table 3.2). The final two treatments compared injection locations, (cranial or caudal; Fig 3.1) using the Lipogen Forte® vaccine for both groups. All injections were dosed at 0.1 ml.

Standard quality control procedures for vaccination were followed, such as vaccination dose calibration at regular intervals, appropriate needle selection (length and gauge), regular needle replacement and vaccine handling and delivery as per manufacturer's instructions. To ensure proper vaccination, five vaccinated fish were euthanized and dissected to confirm appropriate vaccine placement prior to the initiation of standardized vaccination protocols on the rest of the population.

Table 3.2. Vaccination groups assigned to individually PIT tagged Atlantic halibut juveniles. All vaccinations were single intraperitoneal injections.

Group	Vaccine Type[1]	Product Identity	Manufacturer[2]	Adjuvant System	Injection Location
A	Quatravalent	Lipogen Forte®	Aqua Health Ltd., Canada	Liquid emulsion + oil based adjuvant	Caudal I.P
B	Quatravalent	Alphaject 4000®	Pharmaq, Norway	Mineral Oil	Caudal I.P
C	Pentavalent	Advantigen 5.1®	Microtec International Inc., Canada	Mineral Oil	Caudal I.P
D	Saline	Sterile Saline (0.9% NaCl)	n.a.	n.a.	Caudal I.P
E	Non-vaccinated	n.a.	n.a.	n.a.	n.a.
F	Quatravalent	Lipogen Forte®	Aqua Health Ltd, Canada	Liquid emulsion + oil based adjuvant	Cranial I.P
G	Quatravalent	Lipogen Forte®	Aqua Health Ltd, Canada	Liquid emulsion + oil based adjuvant	Caudal I.P

n.a = not applicable

[1] dose = 0.1ml

[2] Vaccine labels and instructions can be found in Appendix 3.

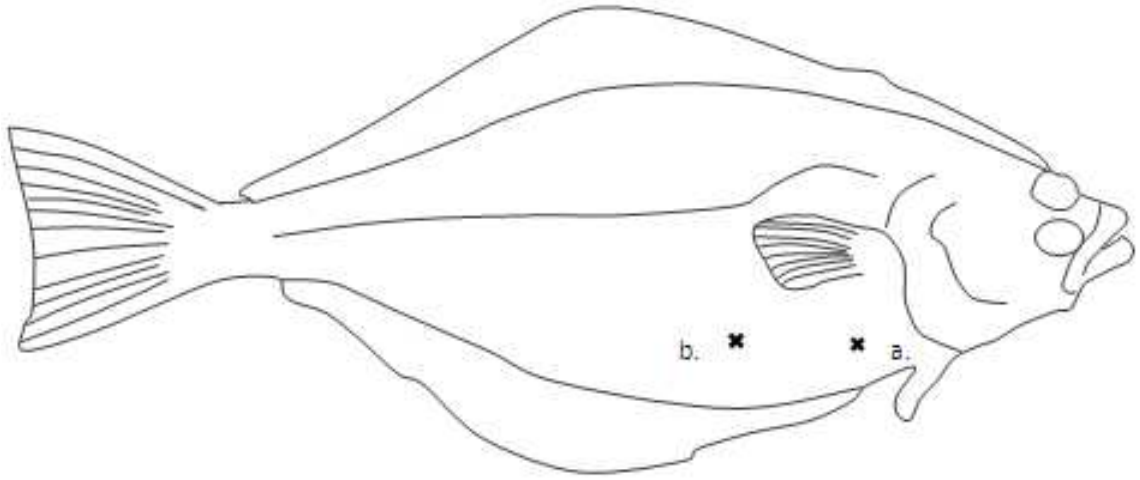

Fig. 3.1.Cranial (a.) and caudal (b.) vaccine injection locations tested for Atlantic halibut juveniles.

3.2.2.1 Farm and management

Water temperature ranged from 8.0 to 13.3°C at the hatchery and 0.5 to 14.5°C at the sea cage site over the course of the study. The fish remained at the hatchery until day 63 or 65 when they were transferred to the marine site in two shipments. The fish accumulated approximately 650 °C-days (DD) post-vaccination by the time of transfer, exceeding the 400 °C -days recommended by each of the vaccine manufacturers. The fish were transferred to a commercial marine site (Appendix 1) previously stocked with Atlantic halibut. The study population was stocked into a single 70 m circumference Polar Circle cage modified with a flat panel Dyneema® bottom tensioned to a circular ring filled with sand, similar to other cages at the site.

3.2.3 Field monitoring and sampling

3.2.3.1 Mortality

Mortality dives were conducted weekly (or biweekly during periods of low mortality) and were attended by study personnel. All mortalities collected from the study population were necropsied, and adhesions scored on each individual fish.

3.2.3.2 Evaluation of intraperitoneal lesions

Adhesions were evaluated post-mortem using a semi-quantitative ordinal scale (Fig. 3.2) modified from the Speilberg Score (Midtlying et al.1996). The serosal surfaces were also inspected for melanin deposits. Adhesions were scored at three distinct time points during the study. On day 190 of the study, a systematic random sample of 371 halibut was obtained from a subset of 2712 mortalities that resulted from a production

incident (i.e., non-infectious disease). The second sample came from mortalities collected weekly over the entire study period, and the third, largest sample collected during the final harvest. Overall, 1949 fish were assessed (vaccinated and controls).

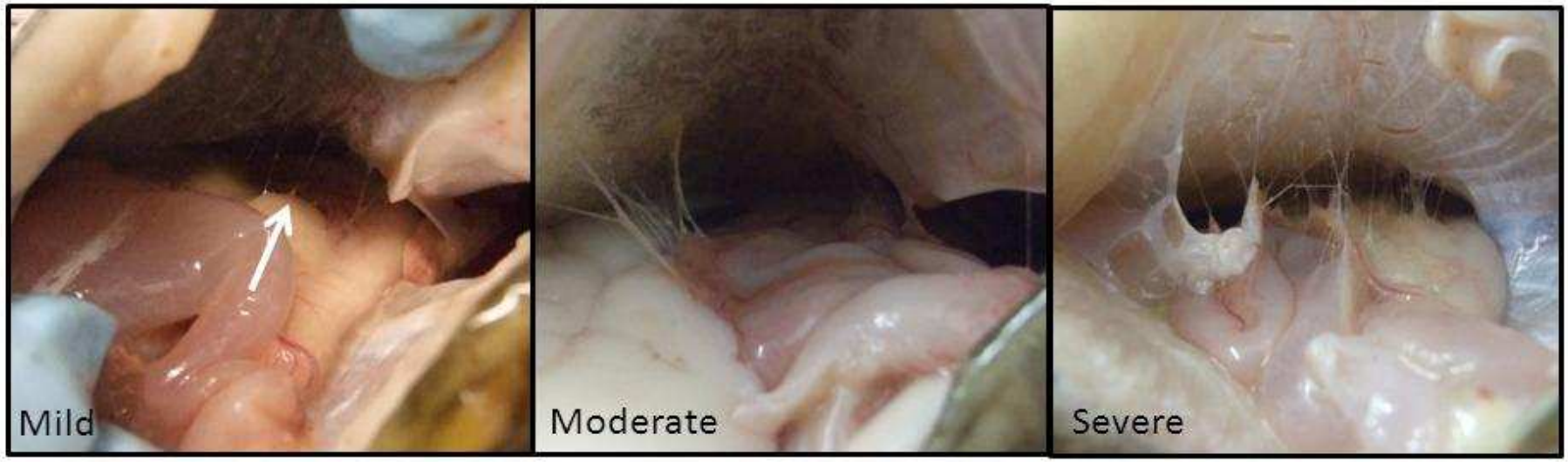

Fig. 3.2. Examples of intra-abdominal adhesions in Atlantic halibut scored using a visual assessment scale (absent not shown).

3.2.4 Statistical procedures

3.2.4.1 Univariate methods

The relative percent survival (RPS) was calculated using the formula, RPS = 100 x (1- [% mortality in vaccinated group / % mortality in baseline reference group]). The saline control group was used as the baseline reference to compare the survival of the different vaccine groups.

The severity of intraperitoneal adhesions scores among vaccination treatments was analyzed using a Kruskal-Wallis non-parametric test. Pre-planned pairwise comparisons of the vaccination treatments were analyzed using a Wilcoxon rank-sum non-parametric test with precise *P*-values calculated using a permutation test using 10000 iterations. The *P*-values of the pairwise comparisons among the vaccine treatments (Lipogen Forte, Alphaject, Advantigen) were adjusted for multiple comparisons using a Bonferroni correction. Differences in adhesion prevalence among vaccine treatments were analyzed using the binomial process and 95% confidence intervals were calculated.

3.2.4.2 Multivariable methods

The individual fish characteristics such as sex, eye migration and cataracts previously identified as important predictors of productivity (see Chapter 2) were controlled for analytically. The productivity data was six sequential time points with outcome variance increasing over time. Seasonal changes explain the large variation in productivity between the periods. These characteristics suggested that a non-stationary

covariance structure would best explain the correlation of observations at the fish level as the time series progressed, while at the same time accommodating the increasing variance. The number of bands for the correlation matrix was determined by incrementally increasing the bands until a completely unstructured matrix was fit or optimal model fit was reached, as determined by the Akaike Information Criterion (AIC: Dohoo et al. 2009a).

The transformation of the outcome weight (W) was necessary so that model residuals followed the assumption of normality. Initial weight (W_0) was run as a covariate for the outcome W. At the end of the first period and at harvest, pairwise comparisons among vaccine treatments (Lipogen Forte, Alphaject, Advantigen) were adjusted for multiple comparisons using a Bonferroni correction.

3.2.4.3 Survival analysis modeling

A Cox proportional hazard survival model was used to analyse survival among the vaccine groups over the study. The data were split at each exit point so that interactions with time could be considered to correct for non-proportional hazards (as described in Chapter II). The saline control was used as the reference comparison group. The best fitting model was constructed with the predictor vaccine group forced into the model. The proportional hazard function was observed graphically to evaluate proportionality and tested using a global test (Dohoo et al. 2009b). A Wald test was used to determine the overall significance of the vaccine group treatments.

3.3 Results

3.3.1 Growth

Significant differences in weight gain were observed among vaccine groups over the first 189-day period following vaccination (Fig. 3.3). No significant difference in weight gain was noted between the two control groups (χ^2_1=1.23, *P> 0.05*) or between the two different vaccine placement locations (χ^2_1=0.14, *P >0.05*) during the first growth period. The differences that developed during the first 189 days of the trial abated over the grow-out period, with no significant differences in growth observed between the three oil-adjuvant vaccines (Fig. 3.4), the two control groups (Fig. 3.5) or the two vaccine locations (Fig. 3.6). At harvest, no significant differences in final weight were observed between any of the treatment groups (χ^2_5=10.95, *P> 0.05*) (Fig. 3.7).

3.3.2 Survival

There were no significant differences in survival among the vaccine groups (Table 3.3; χ^2_4 =2.97, P>0.05). The hazard functions for the vaccine groups did not change over the progression of the study (i.e., no interaction with time).

3.3.3 Side-effects

The prevalence of adhesions differed significantly among the vaccination groups (Table 3.4). Halibut that received an oil-adjuvant vaccine had a significantly higher prevalence of intraperitoneal adhesions compared to either control group. The average severity scores of intraperitoneal adhesions differed significantly among the vaccine groups (χ^2_6 =88.2, *P*<0.01) (Table 3.4). Although the overall number of severe adhesions

was small, the Alphaject group had four times the number of adverse adhesions (n=19) compared to Lipogen (n=4) or Advantigen 5.1 (n=5) groups. Severe adhesions were not recorded in either control group (saline or non-injection). The majority of adhesions were focused in the area immediately surrounding the injection site and involved the attachment of intestines to the body wall.

Table 3.3. Percent mortality and relative percent survival (RPS) in each of the vaccine groups at the completion of the trial.

Vaccine	% Mortality	RPS[a]
Lipogen Forte®	6.0	-15.4
Alphaject 4000®	4.7	9.6
Advantigen 5.1®	4.3	17.3
Saline	3.6	30.8
Control	5.2	-
Caudal	3.3	36.5
Cranial	6.4	-23.1

[a]No differences significant

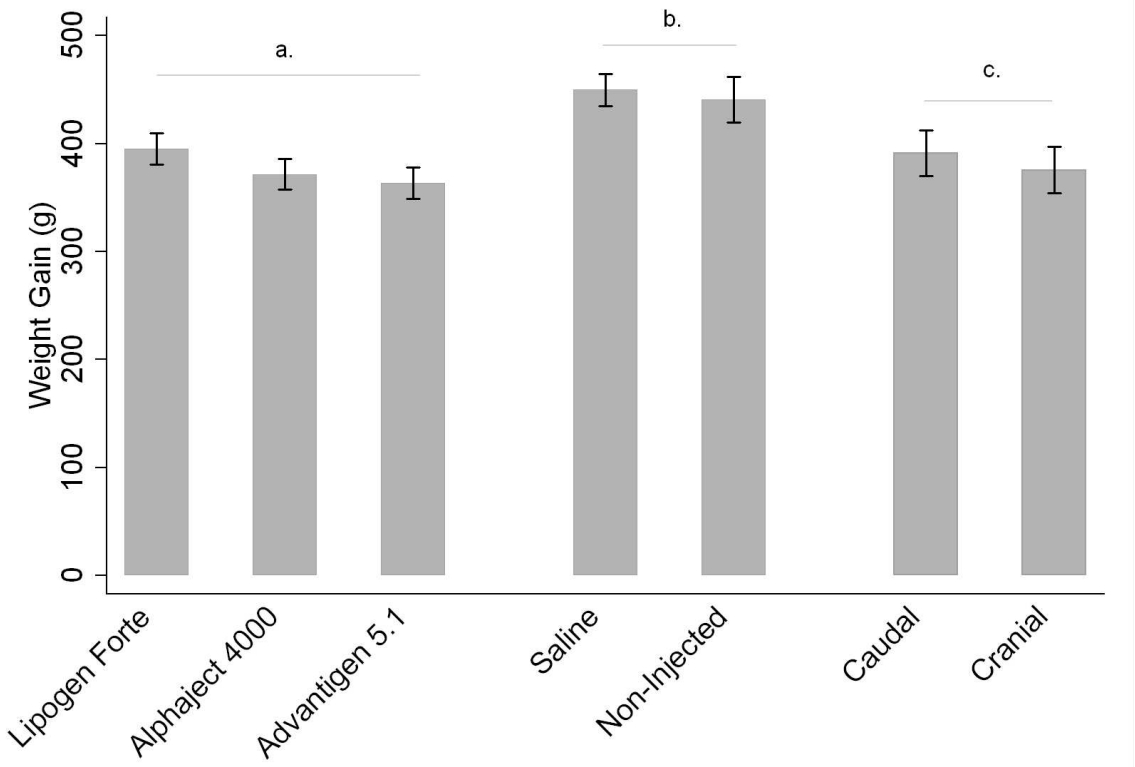

Fig. 3.3. Mean growth during the first growth period of Atlantic halibut receiving one of seven different vaccination and control treatments. Values are mean (± 95% CI). a) Pre-planned comparisons among the vaccinated groups (adjusted for three comparisons) in the first growth period found Lipogen Forte vaccinated fish had significantly better growth than Advantigen (χ^2= 16.9, P_{corr} <0.001), but no significant difference between Lipogen and Alphaject (χ^2= 9.4, P_{corr} >0.05) or Alphaject and Advantigen (χ^2= 1.1, P_{corr} >0.05). All three vaccinated groups were independently significantly different (P_{corr}<0.05) from pooled saline-injected and non-injected control groups. b) No significant difference between saline-injected vs. non-injected controls (pre-planned comparison) (χ^2= 1.23, P>0.05) c) No significant difference between caudal vs. cranial injection (pre-planned comparison) (χ^2= 0.14, P>0.05). (n=3289)

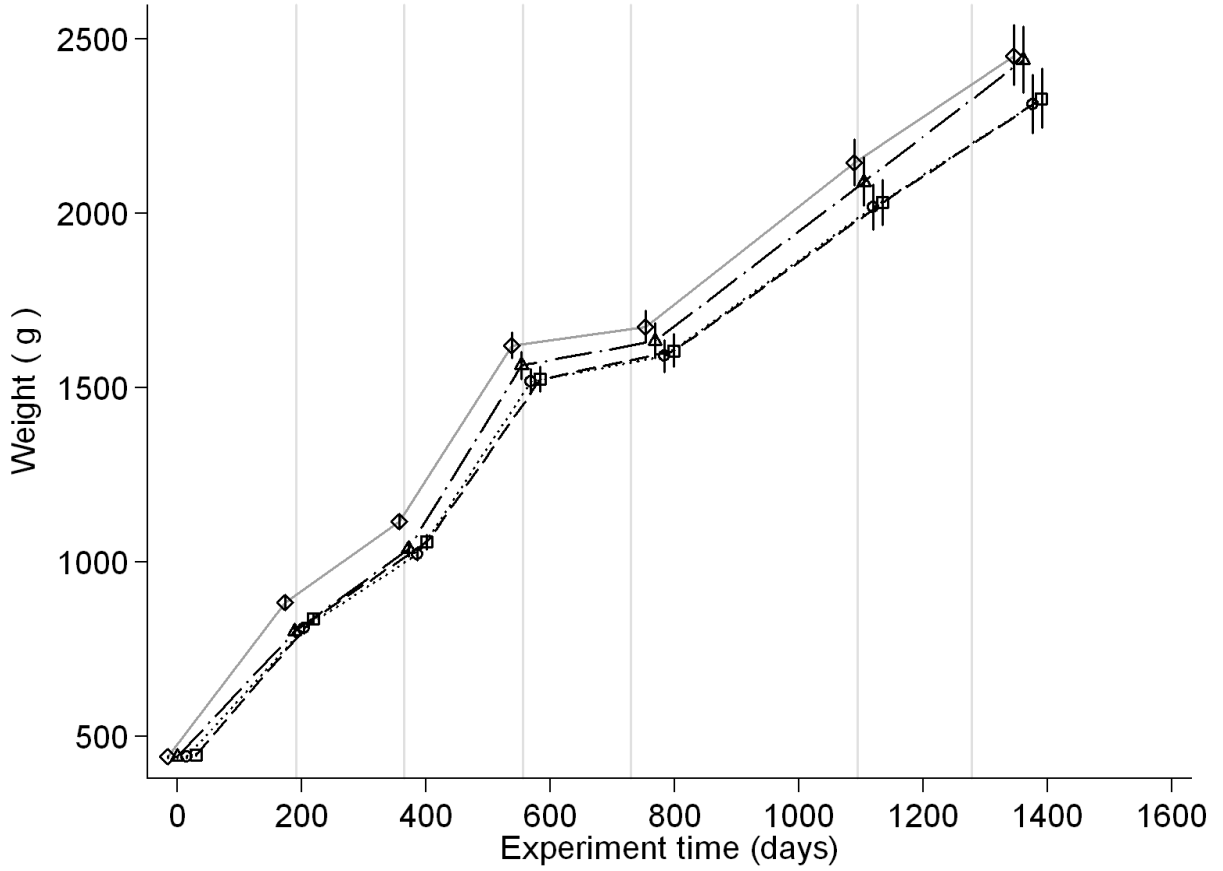

Fig. 3.4. Mean weight of saline-injected controls (solid line) and oil-adjuvant vaccines over the entire study period: Lipogen Forte (dash), Alphaject 4000 (dot) and Advantagen 5.1 (long dash dot). Values are mean (± 95% CI). All groups were sampled on the same dates, but data have been offset for clarity.

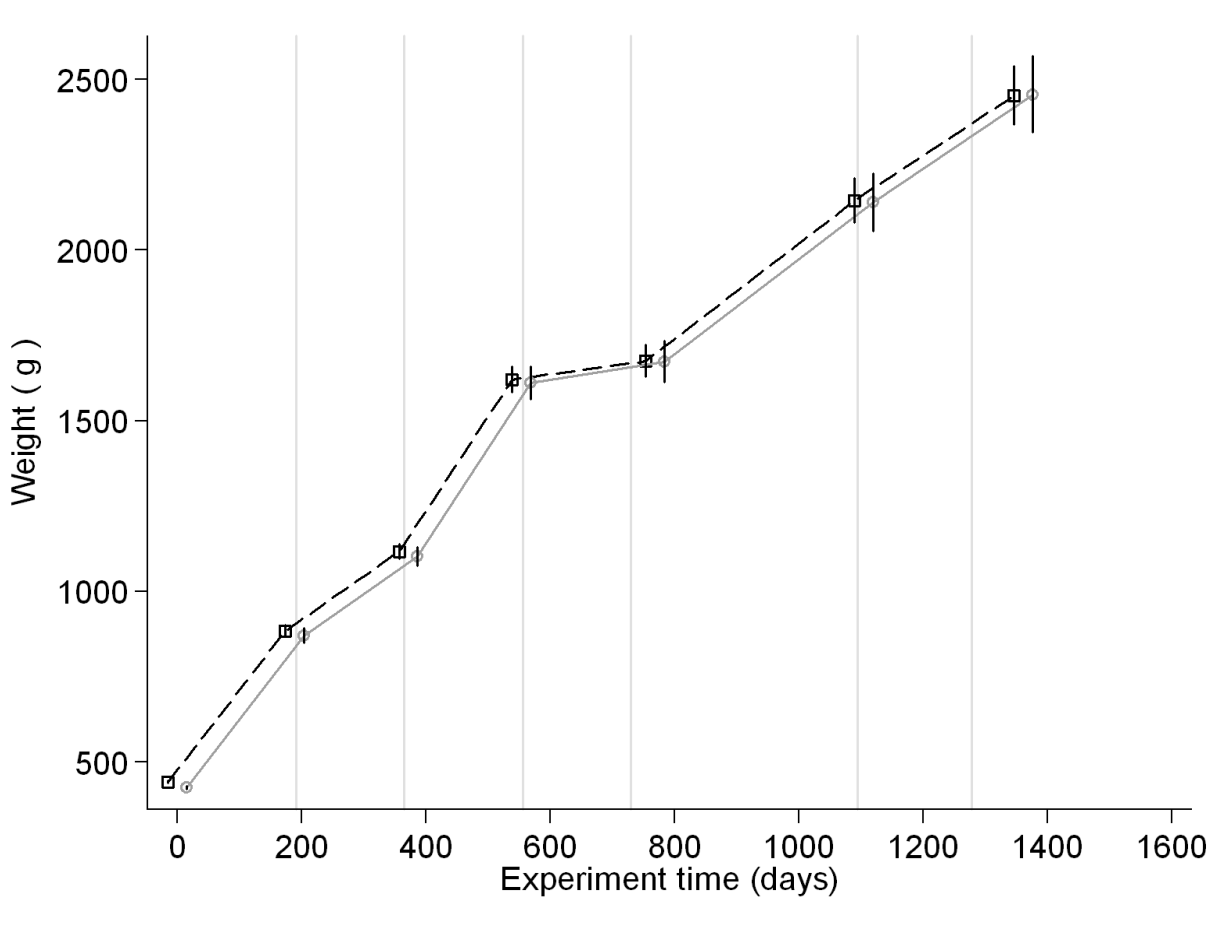

Fig. 3.5. Mean weight of non-injected (dashed line) and saline-injected controls (solid line) over the study period. Values are mean (± 95% CI). Both groups were sampled on the same dates, but data have been offset for clarity

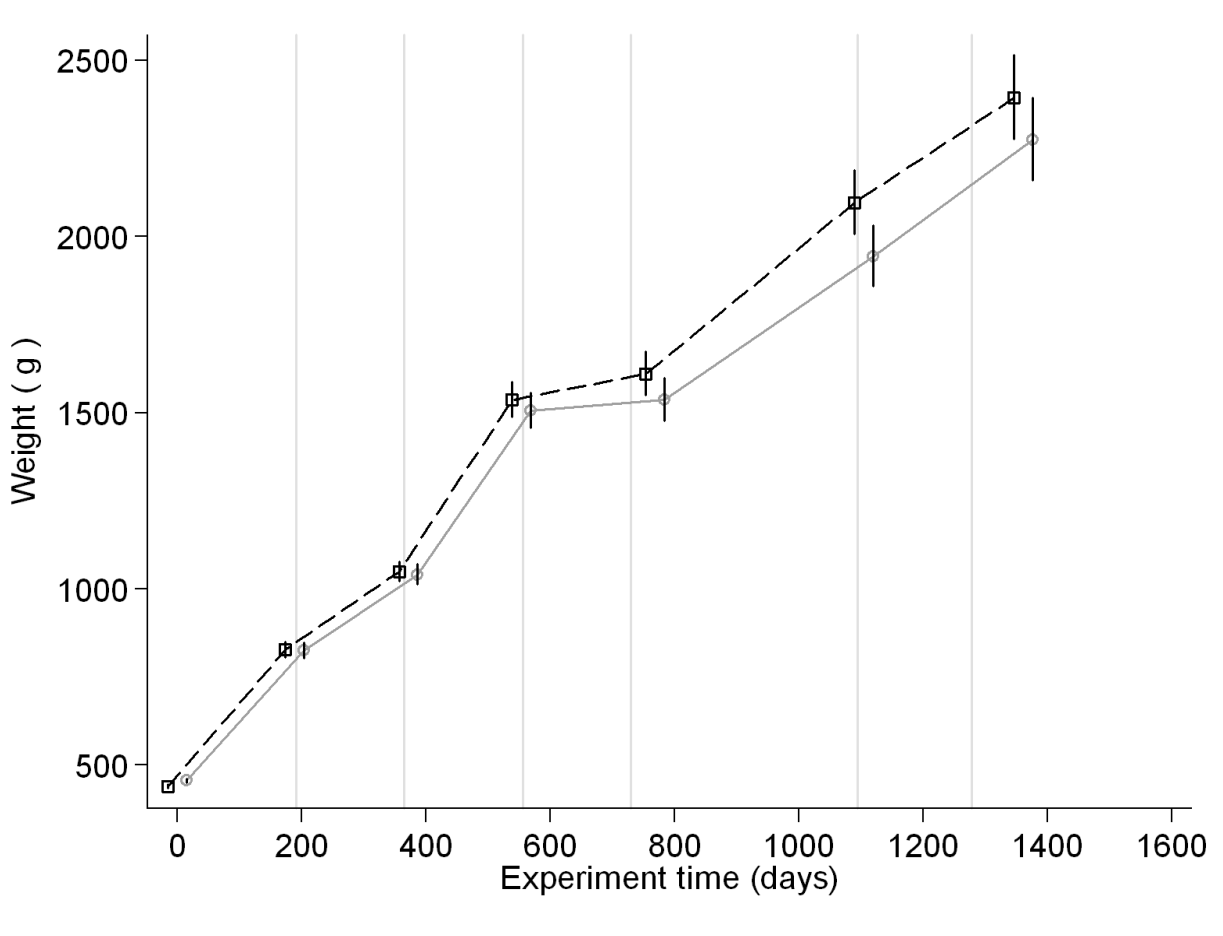

Fig. 3.6. Average weight of Atlantic halibut receiving i.p. Lipogen Forte vaccine in caudal (dashed line) or cranial (solid line) locations over the study period Values are mean (± 95% CI). Both groups were sampled on the same dates, but data have been offset for clarity.

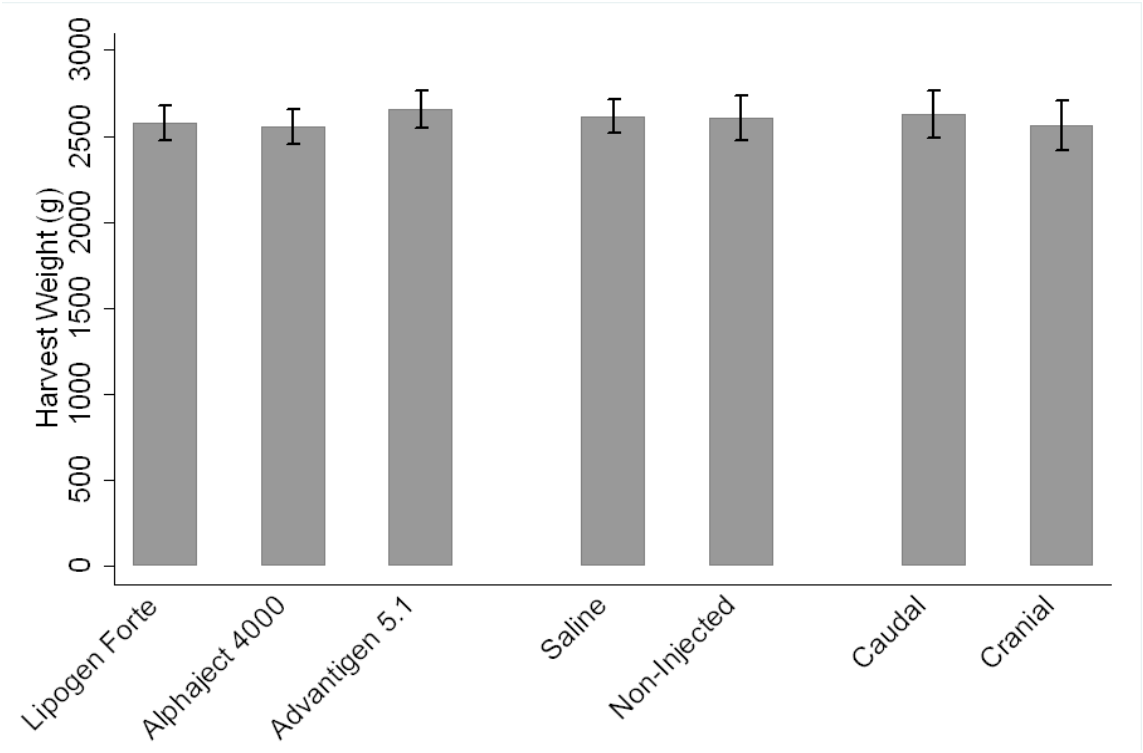

Fig. 3.7. The average final harvest weight of fish in each vaccine treatment. Values are mean (± 95% CI) No significant differences were observed (P_{corr} >0.05). (n=1789)

3.3.4 Health events

During the routine sampling on day 554 of the study, some fish presented with small ulcer lesions on the caudal peduncle and laterally towards the outer fins on the ocular side. Ulcers varied in number and severity, ranging from some fish just starting to show signs of the condition, to others with open lesions or lesions beginning to heal. This was the only time point such lesions were observed with no observed link to mortality. Atypical furunculosis (*Aeromonas salmonicida*) was isolated from two halibut in a neighbouring cage towards the completion of the study. The timing of this discovery coincided with a period of slightly elevated mortality on the farm; however, routine necropsy assessments of mortalities (which included bacterial cultures of kidney) failed to identify clinical signs or positive cultures of this pathogen. Therefore disease challenge could not be confirmed.

Also on sampling day 554 of the study, a lice infestation (suspected, *Caligus elongatus*) was observed. Infestations were moderate with one to four lice per fish on less than half the fish observed. No physical damage was observed on any of the fish. Sea lice were not observed again at any other time in the study.

Table 3.4. The prevalence of intraperitoneal adhesions and the average adhesion severity scores for vaccinated and control Atlantic halibut.

Vaccine Treatment	Sample Size	Adhesion Prevalence (95% CI)	Average Adhesion Severity
Lipogen Forte®	411	24.3 (20.2- 28.8)	0.3 a
Alphaject 4000®	395	35.7 (30.9-40.7)	0.5a
Advantigen 5.1®	359	31.5 (26.6-36.6)	0.4a
Saline	411	15.1 (11.7-18.9)	0.2b
Control	466	16.5 (13.2-20.3)	0.2b
Caudal	209	23.4 (17.8-29.8)	0.3c
Cranial	202	30.7 (24.3-37.6)	0.4c

a) Pre-planned comparisons between treatments (adjusted for three comparisons) showed Lipogen Forte to be significantly less than Alphaject and Advantigen ($Z = -3.96$, $P<0.01$ & $Z = -2.55$, $P =0.05$, respectively). In addition, all three were significantly different from the pooled control group (saline and non-injected control).

b) No significant difference between saline and. control (pre-planned comparison) ($Z = -0.61$, $P >0.05$)

c) No significant difference between caudal and. cranial (pre-planned comparison) ($Z = -1.896$, $P >0.05$)

3.4 Discussion

3.4.1 Growth

Reduced growth for a short time immediately following vaccination has been reported in studies involving Atlantic halibut (Gudmundsdottir et al. 2003; Ingilae et al. 2000) and other species (e.g., Arctic charr *Salvelinus alpinus*) (Pylkko et al. 2000). The period of reduced growth can at least partially be attributed to a period of reduced appetite that commonly follows vaccination. Compensatory growth in the following 6-12 months typically makes up for this lost productivity (Midtlyng et al. 1996; Mutoloki et al. 2004). Compensatory growth is a common occurrence in fish when conditions or ailments that compromised growth are corrected and fish can catch up to their original cohorts (Ali et al. 2003). For this reason, it is suggested that vaccine side-effect studies should last considerably longer than three months so that compensatory growth following vaccination can be observed (Pylkko et al. 2000). This study evaluated the impacts until harvest, allowing the interpretation of vaccine side-effects to the point in time when they are realized by producers in the form of final product value. If vaccinated fish are able to compensate for lost growth in earlier periods by the harvest date, as observed in this trial, then growth reduction is a moot point. However, when growth differences exist between vaccines at harvest, producers should re-evaluate vaccination management plans, as those decisions have financial impact.

Inflammation at the site of injection is thought to cause the fish discomfort; this combined with the stresses of handling likely contributes to the reduced appetite following vaccination (Mutoloki et al. 2004). The protection afforded by an effective vaccine will generally outweigh a slight growth reduction.

The study population was the only cage on the farm with vaccinated fish. Differences between the study cage and the remainder of the farm were unavoidable and included a lower density, different stocking times, and location within the farm lease. Therefore, the comparison of growth across different cages cannot be done reliably within the farm. The conditions experienced by the vaccine groups within the study population were identical to one another and therefore the internal validity of the study is reliable. Anecdotal observations suggested that fish in the study cage had better growth and survival than those in other cages on the farm, as evidenced by the lowest non-specific mortality and highest growth performance over the grow-out as reported in farm records (Per. Comm. G. Skip Wolf).

3.4.2 Side-effects

The majority of intra-abdominal adhesions were mild to moderate adhesions similar to those described in previous laboratory-based vaccination studies on Atlantic halibut (Gudmundsdottir et al. 2003; Bowden et al. 2003). A small minority of the vaccinated halibut developed moderate to severe peritoneal adhesions in which internal organs were firmly attached to abdominal wall by a series of non-transparent membranes. In all of these cases, the fish had received an oil-adjuvant vaccine, reflecting a strong likelihood that vaccination was at least a component of the cause. The

observed adhesions did not result in downgrading or difficulties eviscerating the fish at harvest.

Adhesions were focused in the area immediately surrounding the location of injection, suggesting that the vaccine components remain close to the injection site, as reported in rainbow trout (*Oncorhynchus mykiss*) (Rønsholdt & McLean 1999). This is likely an advantage as adhesions located near the two injection locations are less likely to result in adhesions around organs and sensitive structures that could have significant consequences if adhesions developed there. Although rare, certain vaccine formulations have been found to migrate dorso-cranially in Atlantic salmon, resulting in severe adhesions around the esophagus. These lesions became evident and troubling when the feed size was increased on the farm to accommodate the increased size of the fish. The salmon were unable to pass the larger feed through their esophagus thus resulting in poor growth and ultimately starvation (Lars Spielberg Pers. Comm.).

The exact location of injection, the size and origin of fish, rearing temperature and fish species have been suggested as factors affecting the prevalence and severity of vaccine side-effects in Atlantic cod (Hamid 2003) and Atlantic salmon (Poppe & Breck 1997). In this study, we were unable to distinguish a difference in production between the cranial and caudal injection locations. Both injection locations resulted in similar, low severity of adhesion scores. However, vaccinating caudally was considered easier for practical handling and consistency, as commented by the single right-handed vaccinator employed for the trial. The severity of peritoneal adhesions may be influenced by the time of year during vaccination, vaccine formulation, water temperature and fish condition (Berg et al. 2006; Berg et al. 2007) and the impact of

these factors should be further investigated for Atlantic halibut under commercial conditions.

In a previous study (Ingilae et al. 2000), the vaccination of very small halibut (15g) was found to seriously impact growth in the first 10 weeks post-vaccination. However, compensatory growth in the five months post-vaccination removed size differences among the vaccine groups, leaving the investigators to conclude vaccination under laboratory conditions does not compromise overall growth. Vaccinating larger halibut (i.e., 400 g) seems to afford no extra advantage in terms of growth (Bowden et al. 2003). However, the increased size (up to a certain size) of the fish at the time of vaccination may make i.p. injection easier to accomplish.

The true impact of adhesions is measured by their biological impact (growth/mortality) (Aunsmo et al. 2008). Although statistically significant differences in adhesion scores were observed between vaccinated and control groups and among the vaccine groups themselves, these differences are not biologically important as no significant differences in harvest weights was observed and vaccine adhesion did not result in downgrading of final product. The dataset was additionally explored for associations between adhesion scores and growth (regardless of vaccine group) to further investigate the biological impact of adhesions. No statistically significant associations were observed (not presented). The results of this trial suggest the biological and economic impact of oil-adjuvanted vaccine related adhesions are not a concern to producers at this time.

3.4.3 Survival

Vaccination was not found to influence halibut survival over the course of the study. The saline injected control group had the lowest mortality of all treatments at the conclusion of the trial, but these differences were not statistically significant.

Natural pathogen challenges during production are unpredictable and therefore provide serious difficulties for assessing the vaccine efficacy in clinical field trials. Field trials are at the mercy of the surrounding environment when it comes to disease challenge. Understandably, producers are unwilling to purposefully expose their stock to a pathogen and, even so, such drastic measures are unlikely to reflect true disease transmission under a normal production setting. Atypical furunculosis (*Aeromonas salmonicida*) was identified in one of the surrounding cages at the farm during the study. Throughout the grow-out period, all attempts to isolate the bacterium from mortalities and lethal samples using kidney swabs during regular necropsies were negative. Additionally, there were no changes in the mortality patterns within the study population prior to or following the identification of the pathogen in a neighbouring cage. All three vaccines tested contained antigens for *Aeromonas salmonicida*. The testing of a similar vaccine (Alphaject1200) in Atlantic halibut was demonstrated to be effective against *Aeromonas salmonicida* in laboratory trials (Gudmundsdottir et al. 2003). The lack of differential mortality between the different vaccines groups suggests that although the disease was identified on the farm, it was unlikely that study population was exposed to the pathogen. In the absence of a challenge, the outcome of this field trial is well suited to evaluate the side-effects of oil-adjuvant vaccines in commercial culture.

Each fish had an equal probability of receiving any one of the vaccines, resulting in each fish having equivalent probability to grow and survive in the absence of a pathogen challenge. Although this particular trial did not provide evidence for quantification of vaccine-induced protection, it demonstrated that negative consequences of vaccination (i.e., side-effects of the vaccines and procedures) that are to be expected when disease challenge is absent did not affect growth and survival.

3.4.4 Health

Caligus elongatus commonly infest Atlantic salmon in the region the study took place (Westcott et al. 2004). Observations of *C. elongatus* have been previously reported for Atlantic halibut (Johnson et al. 2004). *C. elongatus* is not host-specific and is commonly observed on other marine species such as Atlantic cod during the fall months in regions with similar conditions (Øines et al. 2006). Although typically regarded as only a minor pest in Atlantic Canada, infestations of this parasite in Norway are reported to cause problems in Atlantic cod (Nygaard 2005) and Atlantic halibut (Bergh et al. 2001). These infections have been treated successfully with organophosphates (Johnson et al. 2004). For this reason, it is recommended that halibut producers in Atlantic Canada conduct samplings on their fish and record settlement numbers during the fall months as the cage-culture Atlantic halibut industry develops in the future.

3.5 Conclusion

In conclusion, a full evaluation of vaccination efficiency must be contrasted against any negative outcomes in order to truly quantify the benefits of vaccination (Thorarinsson & Powell 2006). The results of this study indicate that oil-adjuvant

vaccines have minor biological side-effects in the absence of pathogen exposure. Furthermore, a caudal intraperitoneal injection is preferred because of ease of application but a cranial i.p. injection location is also possible. Overall, fish welfare does not appear to be negatively compromised by vaccinating halibut with oil-adjuvanted vaccines, based on the lack of side-effects observed.

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Chapter IV: EVALUATION OF EXTERNAL OPERCULUM LOOP TAGS TO INDIVIDUALLY IDENTIFY CAGE-CULTURED ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.) IN COMMERCIAL RESEARCH TRIALS

Abstract

The growth, survival, and tag retention of double-tagged (external FT4 lock-on (FT4) and internal passive integrated transponder (PIT)) tagged Atlantic halibut (*Hippoglossus hippoglossus*) was compared to internal PIT tagged controls in a randomized trial. The objective was to assess the suitability of these tags for monitoring the performance of individual halibut in longitudinal trials under commercial cage-culture conditions in the lower Bay of Fundy, New Brunswick, Canada. FT4 tags were chosen due to their similarity to tags used by investigators to track halibut in the wild. A subset of the population randomly received an external FT4 tag inserted through the operculum and were monitored over 1105-day period. The specific growth rate of FT4 tagged halibut was significantly reduced in the first sea summer with no significant difference observed for the remainder of the trial. The differential growth in the first sea summer created a relative size advantage, permitting controls to increase in size significantly faster than FT4 fish in all subsequent periods. FT4 tags did not significantly influence survival under normal commercial cage-culture conditions. However, results suggest that the survival of FT4 tagged halibut may be compromised during stressful

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handling events. Tag retention of FT4 tags was acceptable with 76% of tags remaining at the end of the 1105 day trial. FT4 tags proved to be an effective method to identify individual Atlantic halibut; with one caveat that they seriously bias productivity measures in commercial research trials.

4.1 Introduction

The ability to uniquely identify fish is an important component of fisheries and aquaculture research (Hilborn et al. 1990). A wide variety of individual identification tags are available, and can be categorized into two major groupings: external and internal tags (Navarro et al. 2006), both being commonly used in aquaculture research trials. When selecting a tagging method for aquaculture studies, researchers must take into consideration study objectives, the biology of the fish (Chapman & Bevan 1990; Morgan & Roberts 1976), and tag retention and retrieval requirements. Konstantinov (1978) noted the usefulness of individual fish identification while collecting observations on wild populations of fish. However, the real benefits of individual identification are most realized in aquaculture trials, in which fish are held captive and can be re-sampled with relative ease throughout the production cycle. This allows the use of robust longitudinal statistical methods to determine the effect of fish level treatments (Burnley et al. 2010).

Proper tag choice is critical for unbiased research and several factors must be evaluated when ensuring the chosen tag type is appropriate for the study. First, all potential side-effects on the host should be understood; such as effects on growth, survival, and behaviour (Berg & Berg 1990; Moffett et al. 1997). Knowing the

biological impacts will ensure that data generated from tagged fish can be generalized to untagged populations or will allow for interpretation that accommodates tag effects. Second, logistical aspects of the tags, such as retention, speed of tagging, tag removal, and cost, should be matched to the study objectives (Bergman et al. 1992). Finding a balance between all factors will inevitably require compromise. Cost is frequently used as justification for using inferior tags. It has been suggested that, instead of evaluating the cost per tag, investigators should judge tags based on the cost-per-unit of valid data (Bergman et al. 1992). Tagging costs are typically only a fraction of the total project cost and improper tag selection could compromise study findings and so affect the value of the study (Berg & Berg 1990).

External tags are a low cost option and are easily visible in most situations (Moffett et al. 1997). However, they may cause serious side-effects in the form of reduced growth, survival, and overall health (Roberts et al. 1973; Berg & Berg 1990; Bergman et al. 1992; Moffett et al. 1997). The percutaneous insertion of the tags through the skin is implicated as their main disadvantage (Bergman et al. 1992). Internal tags, particularly small ones, are known to have fewer adverse effects (Nielsen 1992; Astorga et al. 2005). Passive integrated transponder (PIT) tags are often used because they are biologically inert, offer unequivocal recognition of individual fish, and have minimal to no biological impacts as observed in gilthead sea bream, *Sparus auratus* (Linnaeus 1758) (Navarro et al. 2006); brown trout, *Salmo trutta* (Linnaeus 1758) (Acolas et al. 2007); Atlantic salmon, *Salmo salar* (Linnaeus 1758); (Gries & Letcher 2002); and the asymmetric flatfish, olive flounder, *Paralichthys olivaceus* (Temminck & Schlegel 1846) (Lee et al. 2009). PIT tags offer researchers greater generalizability of study

outcomes to untagged individuals or populations as compared to external tags and can be electronically scanned enabling rapid and accurate data collection. The inability to visually recognise fish with a PIT tag requires all fish to be handled in order to identify tagged subjects; this can be particularly difficult in commercial aquaculture settings and requires PIT tagged fish to be maintained separately from non-tagged fish. At harvest, or the conclusion of the study, PIT tags are removed from the abdominal cavity prior to (or during) evisceration without damage to the marketable flesh. This is labour intensive and can be difficult to accomplish in commercial settings.

Identification tags are often used to study commercial fisheries and aquaculture with little knowledge of the tag's effect on the fish (Bergman et al. 1992). External tags have often been used for influential scientific work in Atlantic halibut, *Hippoglossus hippoglossus* (Linnaeus 1758) aquaculture (Bjornsson, 1994; Bjornsson, 1995; Brown 2010) and Pacific halibut, *Hippoglossus stenolepis* (Schmidt 1904) fisheries science (Myhre 1966). However, little attempt has been made to quantify the impact these tags may have on the health and productivity of study fish, compared to non-percutaneously tagged or untagged fish. The use of FT4 lock-on tags (FT4) (Floy Tag Co., Seattle, WA, USA, www.floytag.com) have been recommended for identifying rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792), in commercial aquaculture settings (McAllister et al. 1992) and would appear suitable for identifying asymmetric flatfish species like *H. hippoglossus* for two reasons. The ocular side operculum is prominent and easily viewed from above to allow recapture within aquaculture cages by commercial divers, and the tag is not likely to impede swimming, feeding or opercular movements. In addition, these large tags are quickly and easily removed from fish destined for the food market.

The studies being pursued by the investigators required the tracking of individual fish. Based on the assumption that data from scientific trials utilizing externally tagged fish should account for the potential interfering effects of tags (Berg & Berg 1990), the assessments of external FT4 tags versus internal tagging methods and their impact on growth and survival were instigated. Therefore, the objective of this particular study was to evaluate the retention of FT4 tags, and their influence on growth and survival of juvenile *H. hippoglossus* in commercial cage-culture conditions.

4.2 Materials and methods

4.2.1 Study group and selection

On 14 March 2006, a population of 961 *H. hippoglossus* (mean weight ~400g) held at a commercial fish hatchery were anesthetised using Tricaine methanesulfanate (TMS, Syndel Laboratories Ltd., Qualicum Beach, B.C, CAN,www.syndel.com) and tagged intraperitoneally with PIT tags (Avid Identification Systems Inc. Norco CA, USA, www.AvidID.com). TMS baths were used at all handling points during the study at a concentration of 150 mg/l.

PIT tags were inserted into the peritoneal cavity (Fig. 4.1) through a small incision made by the partial insertion of a 12-gauge hypodermic needle on the blind side of the fish, similar to the process described by Gries & Letcher (2002) for *S. salar.* The trial began 3 May 2006 (day 0) when 483 PIT tagged halibut received an FT4 while 478 PIT tagged fish remained as non-externally tagged controls. Treatment groups were assigned by computer generated random numbers. Following crowding and removal from the rearing tanks using a hand-dip net, fish were placed in anaesthetic baths.

Tagging was carried out by two trained personnel: one scanned and recorded tag information while the second installed the external tag and coated the incision with a Polysporin Triple antibiotic ointment (bacitracin 500 U/g, gramicidin 0.25 mg/g, and polymyxin B 10 000 U/g) (Pfizer Canada, Kirkland, QC, CAN, www.pfizer.ca). FT4 tags consisted of a 14 cm by 0.02 cm laminated vinyl tube each labelled with a unique five digit number. FT4 tags were threaded into a large hollow needle which was then inserted between the preoperculum and the operculum (Fig. 4.1) as demonstrated in American plaice, *Hippoglossoides platessoides* (Fabricus 1780) (Morgan & Walsh 1993). A one-way fastener permanently fixed the tag into a loop. It took approximately 50 seconds to insert the FT4 tag and record the corresponding number on paper records. Controls were handled identically to FT4 tagged fish and held on the tagging table to achieve similar air exposure as FT4 tagged individuals, with the only difference between the two groups being that FT4 fish were pierced and tagged. Water temperature ranged from 8.0 to 13.3°C at the hatchery and from 0.5 to 14.5°C at the sea cage site over the course of the study. The study cage was a 70 m circumference polar circle cage modified with a flat panel bottom tensioned to a circular ring filled with sand. Fish remained at the hatchery until day 63 or 65 when they were transferred to the sea cage site (Appendix 1) in two shipments. Study fish were cohabitated in a single sea cage with 4284 other individually PIT tagged *H. hippoglossus* of the same origin, that were part of an unrelated study.

4.2.2 Field sampling and monitoring

Fish were sampled on days 0, 189, 372, 554, 769 and 1105 of the study. At each sampling, the cage was divided using a large weighted seine to crowd the fish. The

crowded fish were then hand netted with a dip net and placed into the anaesthetic bath for 2-3 minutes. Following sedation fish were measured (weight (W) and length (L_F)), and observed for visible health characteristics. Visual assessment of FT4 tag presence or absence was recorded whenever individuals were handled.

Survival of individual *H. hippoglossus* was monitored by the identification of mortalities recovered by divers on a weekly basis. Day 189 was the first attempt at handling large numbers of *H. hippoglossus* by confining them in a seine net at the sea cage site. Approximately four hours after crowding was initiated, signs of distress were observed and the process was immediately discontinued. The following day, divers recovered the mortalities arising from this unfortunate event. Each dead fish was measured and visually assessed for FT4 tag presence.

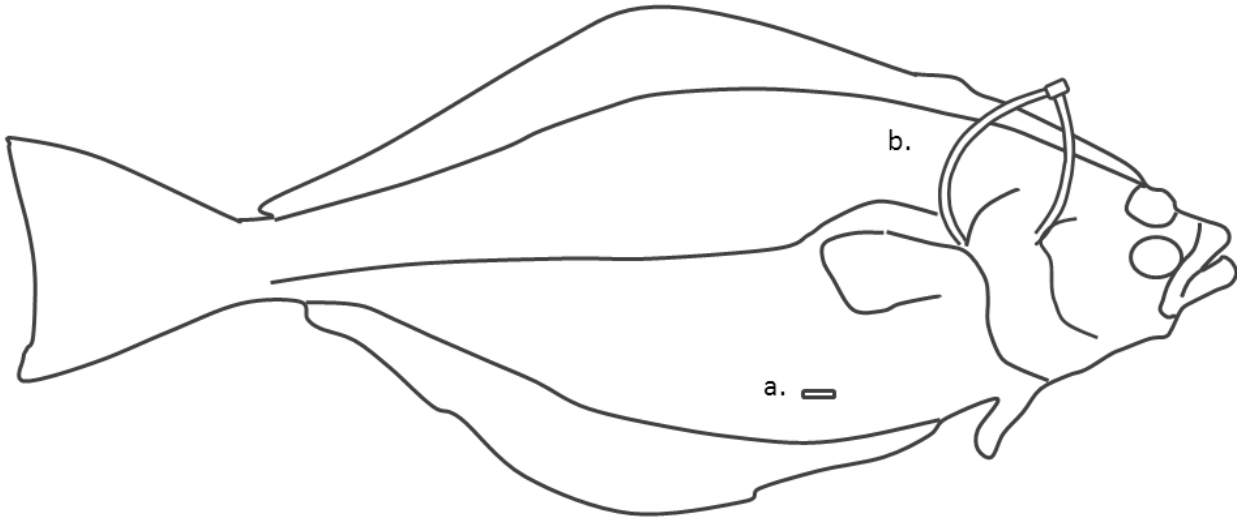

Fig. 4.1. Location of the tags placed on the juvenile Atlantic halibut. (a.) Passive Integrated Transponder (PIT) tag incision location (abocular) permitting insertion into the peritoneum just anterior to the gonads. (b.) Location of FT-4 lock-on tag insertion (ocular) between the preoperculum and the operculum.

Shed tags found on the bottom of the cage were also collected weekly by divers and cross referenced identifying the time of tag loss for individual fish.

4.2.3 Data analysis

All data were analysed using the statistical package STATA 11.0 (STATA, College Station, TX, USA). The W and L_F of FT4 tagged halibut were compared to controls at the beginning of the trial (t_0) using a one-way ANOVA.

4.2.3.1 Productivity

To understand the influence of FT4 tags on fish productivity, four growth performance measures were used: L_F (cm), W (g), proportion increase of initial weight (P) and specific growth rate (G). For the 5 periods between each of the six time points, P was calculated as $W_i W_{i-1}^{-1}$, where W_i is the weight at time t_i, while G was calculated as ($G= ((\ln (W_i) – \ln (W_{i-1})) (t_i-t_{i-1})^{-1}) \times 100$).

Measurements of W, L_F, P and G were statistically analysed using multilevel models that included within fish covariance structures. The variables period and tag type were forced into each model, and their interaction was also included when it improved the model fit significantly. The productivity data comprised a short series of measurements (four and five time points) with outcome variance increasing over time. Seasonal changes explain the large variation in productivity between the periods. These characteristics suggested that a non-stationary covariance structure would best explain the correlation of observations at the fish level as the time series progressed while also accommodating the increasing variance. Model fit was assessed by increasing the

number of bands incrementally until a completely unstructured matrix was fit or optimal model fit was reached, as determined by the Akaike Information Criterion (AIC: Dohoo et al. 2009a).

The transformation of some outcomes was necessary so that model residuals followed the assumption of normality. The W data were natural log transformed (ln) while the square root of the G was taken after the addition of a small value to rescale the outcome to remove all negative numbers that would prevent the transformation. Initial weight (W_0) was run as a covariate for the outcomes W and G to analytically control for any differences in W_0. Transformations were not required for L_F and P with initial length (L_{F0}) and W_0 included as covariates, respectively. The predictive medians and 95% confidence intervals were determined for each treatment over time and used to visualize time points when FT4 fish differed from controls.

4.2.3.2 Survival

A non-parametric Kaplan-Meier survival analysis (Dohoo et al. 2009b) was used to compare the mortality of FT4 halibut to controls over the course of the study. Survival functions were tested using a log-rank test (Dohoo et al. 2009b). Mortalities resulting from the sampling event on day 189 were censored from the survival analysis and compared between groups using a Chi-Square test statistic. Additionally, the relative risk of mortality on day 189 for FT4 fish over controls was calculated.

4.2.3.3 Tag retention

FT4 Tag failures were deemed to occur at the half-way point between when the fish was last observed with a tag and the first observation of the fish without a tag or the recovery of a loose tag from the cage bottom. The predictors W_0 and the order of tagging (surrogate for tagger experience) were tested against survival function of FT4 tags using a semi-parametric proportional hazards model (Dohoo et al. 2009b) to determine if they influenced tag retention. A single estimate of PIT tag retention was determined at the completion of the study by totalling that number of fish that exited the study (death/harvested) and were missing a PIT tag out of the entire cohabitated study population (n=5244).

4.3 Results

4.3.1 Growth impact

Relative to PIT tagged controls, FT4 fish on day zero were significantly ($F_{1, 958}$, $P < 0.05$) heavier (g) x (444.0 ± 10.5 vs. 424.8 ± 11.1) (Mean ± S.E) and significantly ($F_{1,959}$, $P < 0.05$) longer (cm) (35.1 ± 0.3 vs. 34.7 ± 0.3) (Mean ± S.E). However, controls were significantly heavier (g) ($\chi^2_1 = 64.6$, $P < 0.001$) (Fig. 4.2) and longer (cm) ($\chi^2_1 = 169.61$, $P < 0.001$) than FT4 tagged fish at all measurement points past day zero. The outcome L_F was highly correlated to W and followed the same trends. The productivity measures G and P contrasted this trend as these measures were only significantly higher for controls in the first period and not significantly different in all other periods (Table 4.1). The effect of W_0 on G was independent of receiving an FT4 tag (i.e., no interaction between tag type and W_0). A banded non-stationary correlation

matrix (Appendix 4) provided optimal model fit for the data. Regardless of correlation matrix choice (stationary, non-stationary) or analytical method for repeated measures (general estimating equation, multilevel mixed model with specified correlation matrix) the model coefficients and standard errors were robust.

Table 4.1. Median specific growth rate (G) and proportional increase in weight (P) of FT-4 lock-on tagged and control halibut with their respective 95% CI for each growth periods over the course of the study.

		G				P_r			
Period	Season	Control	(95% CI)	FT4	(95% CI)	Control	(95% CI)	FT4	(95% CI)
0-189	Summer	0.35	(0.33-0.37)	0.23	(0.22-0.24)	1.99	(1.94-2.05)	1.57	(1.53-1.61)
190-372	Winter	0.1	(0.088-0.12)	0.1	(0.09-0.11)	1.22	(1.19-1.24)	1.21	(1.19-1.23)
373-554	Summer	0.19	(0.18-0.21)	0.18	(0.17-0.20)	1.44	(1.40-1.47)	1.42	(1.38-1.45)
555-769	Winter	0.02	(0.01-0.03)	0.02	(0.01-0.03)	1.05	(1.02-1.08)	1.05	(1.02-1.08)
770-1105	Both	0.07	(0.07-0.08)	0.07	(0.06-0.08)	1.28	(1.25-1.32)	1.28	(1.24-1.32)

4.3.2 Survival

No tagging or handling mortality was observed in either study group in the 24 hours immediately following the tagging procedure. Mortality in the FT4 group was not significantly different ($\chi^2_1 = 0.17$, $P > 0.05$) from controls over the 1105 day follow up (Fig. 4.3). Of the 53 mortalities that occurred independent of the first handling event, 28 were FT4 and 25 were controls, corresponding to 5.8% and 5.2 % mortality, respectively (Fig. 4.3). Mortality observed immediately following the handling event on day 189 involved 48.9% (455) of all fish remaining in the study, corresponding to 255 (54.7%) and 200 (43.0%) for FT4 and controls, respectively. This being a statistically significant difference ($\chi^2_1 = 13.74$, $P < 0.001$) of 11.7%. Interpreted another way, FT4 fish were 1.28 (95% CI: 1.12, 1.47) times more likely to die compared to controls immediately following this handling event.

Over the course of the study, 98 of 961 (10.2 %) *H. Hippoglossus* became lost to follow-up, of which 33 (34.3%) were attributed to mortality. The remaining 65 were untraceable, with 27 and 38 fish belonging to the FT4 and control groups, respectively. Although these were likely uncollected mortalities, the possibility of escape or poaching (i.e., illegal removal) cannot be excluded. Associations between losses to follow-up were explored between W_0, treatment group or tagging order, with no significant associations found.

4.3.3 Tag retention

Estimates for tag retention in the FT4 group decreased gradually over the course of the study (Fig. 4.4). FT4 tag retention was estimated at 75.0% (95% CI: 70.7, 78.7) by day 1105 of the study, implying that these fish would have been unidentifiable had they not also been PIT tagged. FT4 tag retention dropped sharply from 93.0% (95% CI: 90.1, 94.8) on day 189 to 87.8% (95% CI: 84.5, 90.4) on day 190 after the handling event. Neither, W_0 ($\chi^2_1 = 1.43$, $P > 0.05$) or the order of tagging ($\chi^2_1 = 0.38$, $P > 0.05$) were significantly associated with loss of FT4 tags. Minor, but persistent, lesions were observed in a majority of the FT4 tagged fish. Lesions were typically found around the point of tag insertion, along with mild pressure necrosis along the lateral gill filaments. Thirty-six of the 125 FT4 tags lost were recovered on the bottom of the cage, the majority of these tags were found intact, indicating they had transited through the operculum. Seven fish in the FT4 group where noted to have a missing tag and associated ripped opercula, indicating the tag had transited through the operculum. No significant difference in W_0 was observed between fish with ripped opercula and the remainder of the population (ANOVA, $F_{1,5237} = 0.12$, $P > 0.05$). Of the FT4 tags that were recovered from the bottom of the sea cage the majority (~70%) were found in the first 169 days (prior to the first handling event).

The total estimate of PIT tag retention was determined to be 94.7% (95% CI: 94.1, 95.3). The relationship between PIT tag loss and W_0 was not explored because halibut without PIT tags could not be linked with previously recorded data.

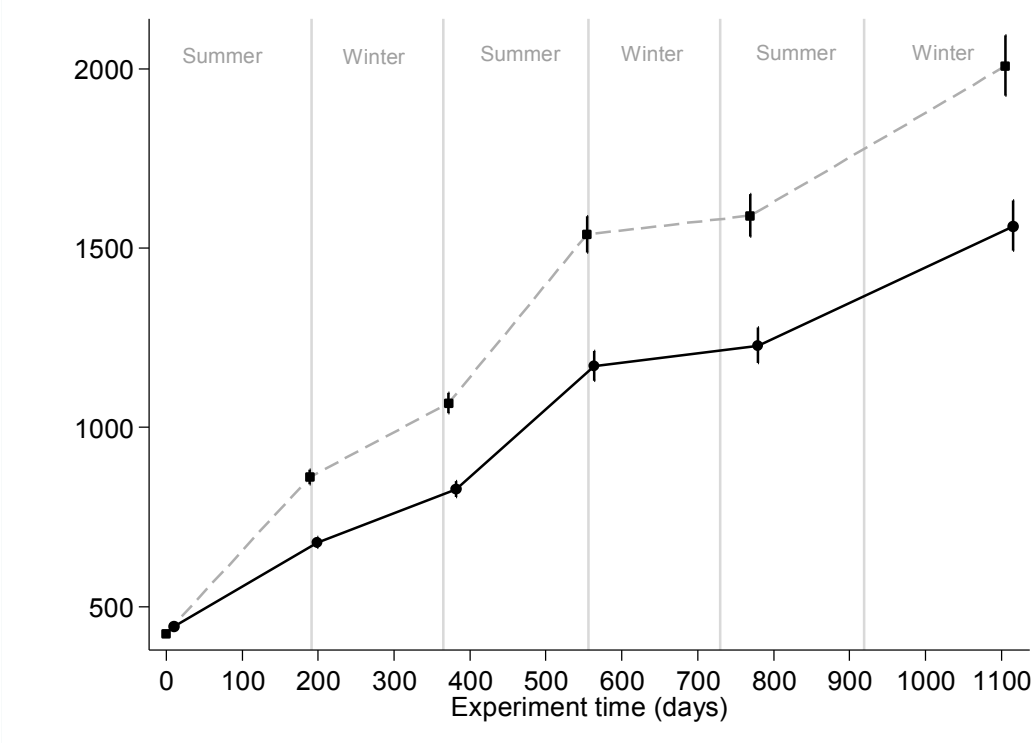

Fig. 4.2. Median weight (W) (g) comparison of Control (■ with dashed line) and FT-4 lock-on (● with solid line) tagged Atlantic halibut as predicted by a mixed model over a 1105 day grow-out period. Error bars represent 95% CI. Tagging took place on 03 May 2006. Both groups were sampled on the same dates, but data have been offset for clarity.

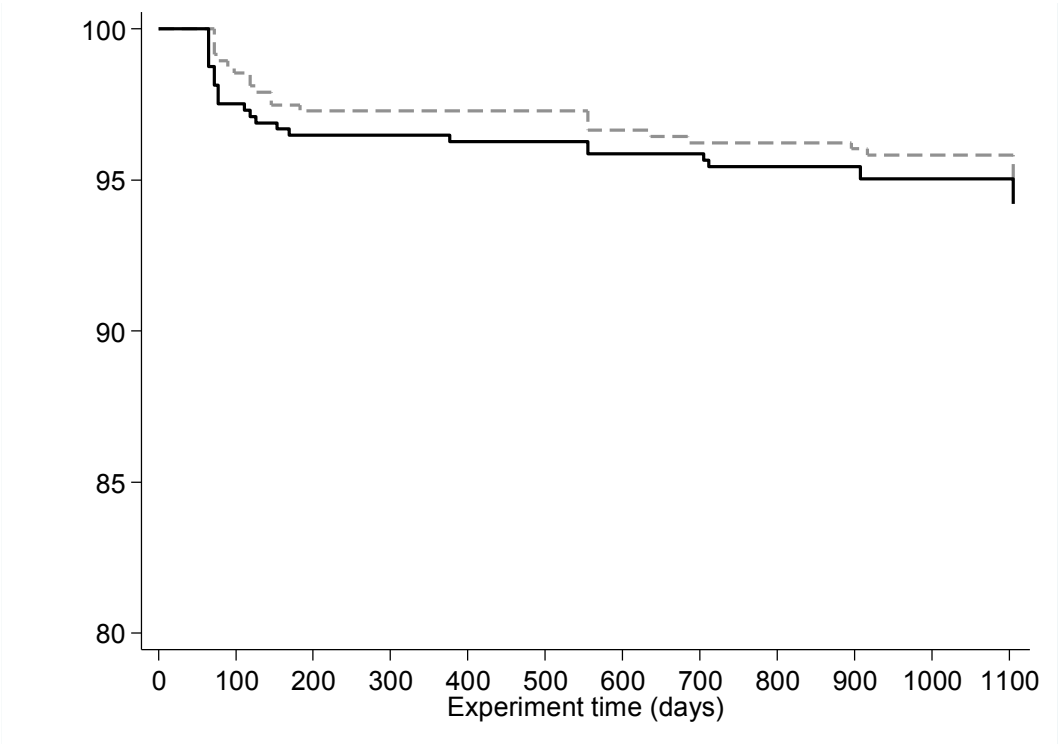

Fig. 4.3. Kaplan Meier survival curve comparing FT-4 lock-on (solid line) and PIT tagged control (dashed line) percentage survival over an 1105 day period.

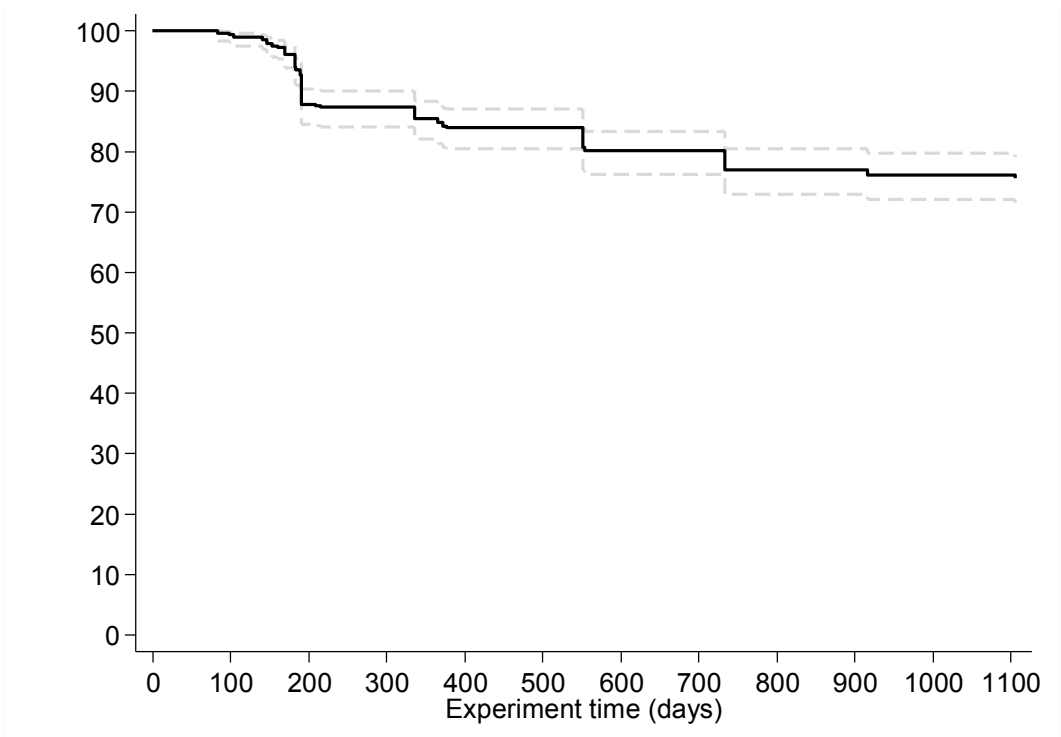

Fig 4.4. Kaplan Meier survival curve of FT-4 lock-on tag retention (solid line) and 95% CI (dashed line) in Atlantic halibut at a sea cage site over an 1105 day period.

4.4 Discussion

Determining the suitability of FT4 tags for the long-term identification of *H. hippoglossus* in commercial aquaculture productivity trials was the objective of this study. Various authors have claimed that percutaneous external tags have serious biological side-effects in the majority of fish species (Bergman et al. 1992). Despite this, the benefits of using external tags in commercial studies are numerous, particularly for allowing identification of research subjects housed within commercial populations thereby increasing the generalisability of the study results to farm conditions. Two non-percutaneous externally visible identification options were considered: visible implant elastomer (VIE) and Panjet. Both of these tagging options have limited to non-existent biological effects in flatfish (Reig et al. 2003; Thedinga et al. 1997). However, they both lack sufficient visibility to enable the fish to be identified and captured by divers.

A variety of important advantages made operculum loop tags an appropriate choice for long-term field studies. FT4 tags inserted through the operculum were not expected to damage the marketable flesh of the fish, which is important in commercial trials in which the fish are typically marketed upon completion of the study. The tags can also be easily identified by divers during the grow-out period and they can be quickly identified and removed at harvest time, or at any point in the study. Finally, there was no obvious hindrance to the fish's normal activities or swimming behaviour.

4.4.1 Productivity impacts

The growth of halibut in commercial cage-culture operations was determined to be negatively influenced by the use of FT4 tags. A multilevel mixed model with a specified correlation matrix was the chosen analytical tool because it considers the correlation of outcomes within a fish over time. The need for a non-stationary correlation matrix was expected from the non-equidistant sampling, and the need to account for the growth stanzas (periods of differing growth) of the fish as they matured.

The highest G was observed between days 0 and 189, while increased G was also observed between days 372 to 554. The periods of high growth included summer months where warmer water temperatures result in higher feeding and metabolic rates. W_0 was found to be negatively correlated with G in the first period. This is explained by the different growth stanzas that occur, with smaller halibut having higher growth rates (Aune et al.1997; Brett 1979). Although a significant difference in W_0 was observed at the beginning of the trial this was not considered a biologically significant difference. Additionally, by using W_0 and L_0 as covariates these differences were controlled for analytically. The significant growth rate reduction of FT4 fish in the first period provided control fish with a relative size advantage which was maintained throughout the experiment. Growth rates in later periods were similar for FT4 tagged and controls but FT4 fish were unable to adopt a higher growth rate to compensate for reduced growth in the first period.

As far as is known, this is the only study to compare the growth of externally tagged flatfish to a non-externally tagged control group. The use of external operculum tags in

H. platessoides was not observed to significantly reduce growth (Morgan & Walsh 1993). However, growth was only compared among externally tagged (percutaneous) treatments. The growth of anadromous Arctic char, *Salvelinus alpinus* (Linnaeus 1758) was determined not to be impacted by (external) Carlin tags inserted near the base of the dorsal fin (Berg & Berg 1990). Similar findings (for tags inserted near the base of the dorsal fin) have been observed in Atlantic cod, *Gadus morhua* (Linnaeus 1758) tagged with data storage tags in both tank and field studies (Righton et al. 2006). This suggests the base of the dorsal fin is superior location for tag placement. However, it should be stated that these studies were conducted on wild fish, in which small sample sizes and a lack of repeated observation over long periods contribute to the potential of unknown confounding factors. Fisheries biologists studying both *H. hippoglossus* and *H. stenolepis* often use wire operculum tags that are inserted around preopercular bone, all within the fleshy portion of the opercular plate (Myhre 1966). The main difference with that method and the one described here being that the tag never enters the gill chamber, preventing the tag from obstructing the operculum and contacting the lateral gill filaments. Although this form of tagging has long been used in wild fish tracking and is likely a superior method, given the small size of the fish at tagging (~400g) an alternative protocol was deemed necessary.

These data were analysed as intent to treat, indicating that once a fish was assigned to a treatment group it was analysed that way for the entire study, regardless of the tag becoming lost at some point during the study. Analysing the growth data this way is expected to minimally bias the growth of FT4 tagged fish towards the null hypothesis.

The results of this study help to quantify the general conclusion that percutaneous tags have considerable impacts on the growth of fish, as reported by Bergman et al. (1992). Previous findings on the impacts of external tagging in other species have shown similar results (McFarlene & Beamish 1990; Stoettrup et al. 2002). Although fish tagged at a smaller size often have increased negative growth side-effects (McFarlene & Beamish 1990; Bergman et al. 1992), no relationship between W_0 and growth reduction was observed. However this may have been due to the comparatively large size at the time of tagging.

Four reasons may have contributed to the growth reduction of FT4 tagged Atlantic halibut as observed in this study:

1) The tag placement around the ocular side operculum may have restricted the movement of the operculum and prevented the operculum from properly sealing on the gill chamber. This may have reduced respiratory efficiency, diverting energy that otherwise would be available for growth.

2) The potential operculum restriction may have compromised the feeding ability of the tagged fish. Adult flatfish capture prey or feed pellets by a combination of suction, jaw protrusion, and ram ingestion (Bels & Davenport 1996). Improper sealing of the operculum on the gill chamber due to the tag may have compromised the suction component of feeding and therefore reduced the feeding efficiency. However, it is unlikely this physical limitation explains all the differential growth observed between FT4 tagged fish and controls. As the study population was fed to satiation throughout the study to avoid hierarchy formation.

3) The physiological cost of maintaining osmotic balance and healing the persistent lesions found around FT4 tags likely contributed to the reduced growth of externally tagged fish. Similar lesions have been observed in other externally tagged fish (Righton et al. 2006; Roberts et al. 1973). External tags become bio-fouled over time (Stoettrup et al. 2002) increasing their weight and abrasive nature. Despite observations that similar lesions in tagged *G. morhua,* did not compromise growth (Righton et al. 2006), it is reasonable to consider that the abrasive nature of FT4 tags were at least partially responsible for the reduced productivity of FT4 tagged halibut.

4) FT4 tags may create a secondary insult on growth rates by attracting aggressive interactions from other *H. hippoglossus* within the farm. Farm staff frequently observed striking and biting of the FT4 tags by other halibut (Kory Leslie, Pers. Comm.). These attacks may result in FT4 tagged fish becoming lower ranking individuals within the population hierarchy therefore reducing growth (Stefansson et al. 2000).

4.4.2 Survival

Chronic tag lesions are reported to increase the fish's susceptibility to infectious pathogens (Roberts et al. 1973). Although lesions created by the FT4 tags did not appear to compromise survival over the normal course of the study, mortalities related to the handling event that occurred on day 189 suggested that FT4 fish were more susceptible to mortality during stressful, hypoxic events than controls. This may be due to the restricted pumping action of the operculum or simply that opercularly tagged fish were chronically stressed and more susceptible to severe, acute stressors.

4.4.3 Tag retention

The FT4 tags and PIT tags exhibited considerably different retention. PIT tags had much higher retention, similar to findings with double tagged Arctic grayling, *Thymallus arcticus* (Pallas 1776) (Buzby & Deegan 2004). Tag retention is influenced by fish size at the time of tagging, with larger fish having higher retention rates (McFarlane & Beamish 1990). We found FT4 tag loss to be independent of initial size possibly because the halibut in our trial were relatively large at the time of tagging. The majority of shed tags were recovered from the cage bottom prior to the first handling event suggesting that the operculum damage occurred most often in smaller fish or perhaps time was required to 'harden' the operculum. Other authors have noted operculum tags transiting through the opercula (Morgan & Walsh 1993; Sanchez-Lamadrid 2001) resulting in lost tags. Sanchez-Lamadrid (2001) identified the small size of the fish at tagging as the reason for this type of tag loss.

Overall, retention was reasonably high, with a tag loss of only 25% over three years. The cost of tagging additional individuals to achieve minimum sample sizes at the end of the study would be reasonable. Compared to other external operculum tags, the retention of FT4 tags in halibut was quite high. For example, in a study by Sanchez-Lamadrid et al. (2001), 66% of *S. aurata* lost their operculum tags within the first 20 days, with 93.2% missing after only 79 days. A separate tank based study using a flatfish species reported similar results whereby 66% of *H. platessoides* lost their operculum tags over a one year study (Morgan & Walsh 1993). Culture conditions are often responsible for variable tag retention (McAllister et al. 1992). Between days 189 and 190, an abnormal number of FT4 tags were lost in the current study. During this period

fish were held in close proximity in a seine with, a large number of mortalities collected the following day for observation. These factors are likely responsible for the observed drop in FT4 tag retention immediately following day 189.

4.4.4 Study limitations

The unfortunate handling event on day 189 of the study that removed approximately 50% of the study population does have important consequences to this study. Assuming a greater proportion of weak fish in the FT4 group, a stressful event like the one experienced during the handling event on day 189 would likely result in a higher mortality in that group. Thus, removing a greater number of the "weaker FT4 fish" therefore the ongoing assessment of surviving FT4 fish actually evaluated "strong, FT4 fish" compared to controls, rather than "all FT4 fish" compared to controls. This would likely bias the difference in productivity and survival measures towards the null. Therefore, we are confident that FT4 tags have considerable growth impact given that significant impacts on productivity were measured despite these biases.

In conclusion, our results agree with the general statement that any tag that passes through the protective barrier of the skin can be expected to negatively impact the "host" (Bergman et al. 1992). It was also suggested by Bergman et al. (1992) that tag effectiveness should be measured in terms of cost per unit of valid data. Based on this criterion, FT4 tags would seem acceptable in terms of tag retention and survival. However, the growth reduction observed indicates that growth data generated from FT4 tagged populations are not representative of untagged populations in commercial culture settings. Although it would be advantageous to use a highly visible external tag that has

no impact on growth, survival and behaviour, this is not a reality. Until such a tag is developed FT4 tags can be used, provided that their impact is quantified and studies account for those effects.

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Chapter V: AN ALTERNATIVE METHOD FOR THE OVERLAND TRANSPORT OF JUVENILE ATLANTIC HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.): IMPACT ON POST TRANSPORT MORTALITY AND ECONOMIC-EFFICIENCY

Abstract

The objective of this study was to evaluate a convenient, low cost modification to conventional salmon smolt transport tanks for the efficient transport of juvenile Atlantic halibut. A controlled trial was designed to estimate post-transport mortality of Atlantic halibut transported using an experimental Stratified Transport System (STS) compared to the traditional Unstructured Transport System (UTS) based on marking a subset of randomized individuals and determining their post-transport outcome. Wire mesh cages were stacked within transport tanks to create the STS, increasing the surface area for settlement and homogeneously distributing the halibut throughout the tank. Utilization of a STS was found to significantly reduce post-transport mortality by 3.1% (95% CI, 0.03%-5.9%). A stochastic cost-benefit analysis determined investment into a STS to be cost effective, with a mean benefit-cost ratio of 1.31 (95% CI, 0.68-2.00) after two years and a mean five year Net Present Value of $85,176 (95% CI, $46,906-$125,630). The implementation of a STS was found to be technically feasible and

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economically-efficient method to improve post-transport survival and overall welfare of transported Atlantic halibut.

5.1 Introduction

Live transport of fish from hatcheries to grow-out sites is a necessary and critical component for most finfish aquaculture operations. To optimize the economic efficiency of transport, transport tanks are stocked to maximal carrying capacities (Portz et al. 2006) while attempting to minimize post-transport mortality. During the transport process fish are exposed to a variety of sub-lethal stressors that include crowding, chasing, air exposure, confinement, agitation as well as changes to temperature and salinity (Carmichael et al. 2001; Harmon 2009; Nomura et al. 2009). The loading and unloading of transport tanks is the most stressful component of the transport process (Iversen et al. 1998; Maule et al. 1988; Robertson et al. 1988). Given the right combination or severity, stressors are capable of cumulative and interactive effects (Carmichael et al. 2001; Maule et al. 1988) compromising the survival of transported stock. For this reason, improvement to any part of the transport process has the potential to improve fish health and reduce post-transport mortality (Pickering 1993).

Teleost fish are known to use nervous, immunological and hormonal mechanisms to adapt to stressors (Barton & Iwama 1991). Adaptation to transport stressors comes with metabolic costs, diverting resources away from normal energy budgets, potentially compromising the health and growth of the fish (Barton 2002). Post-transport mortality is an insensitive but useful indicator of stress in transported animals (Knowles & Warriss 2007). The use of post-transport mortality as an indicator for stress

is rationalized using the stress, distress, mortality model outlined by Moberg (2000), where a severe stressor or the accumulation of mild stressors causes distress in the animal. Should that distress exceed the animal's reserves or capacity to cope, mortality will result. When transporting live fish, delayed post-transport mortality is indicative that stressors associated with transport and acclimation have exceeded the animal's natural ability to cope (Gomes et al. 2003; Portz et al. 2006).

The loss of a small proportion of stock post-transport is a predictable outcome, with an acceptable cut point for losses depending on the species transported, season and equipment used. For instance, the equipment and procedures for transporting Atlantic salmon (*Salmo salar*) are well refined with post-transport mortality consistently around 1-2% of transported stock (Nomura et al. 2009). When transporting juvenile Atlantic halibut (*Hippoglossus hippoglossus L.*) the loss of only 3% of transported stock is considered achievable given current practices (Stuart et al. 2010). However, transport events with unacceptably high post-transport mortality frequently occur. The transport protocols for Atlantic halibut have been developed almost exclusively through industry experience (Brown 2002) and by transfer of knowledge gained from the extensive transport of salmonids. The high cost and limited availability of halibut juveniles as compared to salmon smolt make post-transfer mortality an important factor in determining the profitability of halibut culture.

Once Atlantic salmon are loaded into the transport tanks they begin to recover from loading stressors (Nomura et al. 2009). Similar patterns are unlikely for Atlantic halibut transported using similar transport equipment due to major differences in behaviour and anatomy. By nature, Atlantic halibut are a docile species with

comparatively low oxygen demands and metabolic rates (Brown 2002; Davenport et al. 1990) lending themselves as good candidates for transport. However, once loaded into transport tanks halibut have one of two options: settle or swim. Being an epibenthic species, halibut lack a swim bladder and are negatively buoyant (Gibson 2005). In order to remain in the water column, halibut must actively swim, thereby exerting themselves. For this reason, they are most often found resting on available substrate (Brown 2002). Halibut of a size suitable for transport to cage-culture sites (200-800g) are stocked in transport tanks at approximately 750 Percent Coverage Area (PCA) which can be visualized as the number of fish superimposed on one another in a tank (i.e., 7.5 fish layers). Transport densities are 3 to 4 times greater than what occurs in the hatchery (Brown 2002), but are necessary to make transport economically feasible. Halibut will settle on top of one another, and this is typical under culture conditions. However, aggregations of halibut several layers deep can impede the exchange of water around the fish, creating heterogeneous areas of suboptimal conditions or "dead spots" (Brown 2002; Reig et al. 2007). Dead spots are characterized by one or a combination of the following: reduced dissolved oxygen (hypoxia) (Reig et al. 2007), increased carbon dioxide (hypercapnia) (Moran et al. 2008), and increased ammonia (Harmon 2009). In addition to suboptimal water quality, the increased physical contact between settled fish may result in damage to mucus layers, eyes and fins, potentially compromising immunity and osmoregulatory function (Ross and Ross 2008). Conversely, all or a proportion of the fish can swim to maintain themselves in the water column. Under culture conditions, between feedings, approximately 25% of halibut within sea cages can be found actively moving in the form of brief swims lasting less than 5 minutes (Cordero

Martinez et al. 1994). A recent study demonstrated that 10 minutes of enforced swimming was adequate to completely exhaust cultured turbot (*Scophthalmus maximus*), resulting in a moderate stress response indicated by an increase in blood cortisol levels (Van Ham et al. 2003). Therefore, it is unlikely that one would find a large proportion of the fish swimming at any one time during extended transports. The increased swimming activity and associated stress likely serve to further degrade water quality due to the concomitant increase in metabolic rate (Portz et al. 2006). Even if 25% of halibut were to swim in transport tanks this would result in a PCA of 560% and aggregations that still likely to produce the suboptimal conditions previously noted. Transportations overland by truck are particularly challenging in contrast to boat transfers because fish must be maintained in a limited, static volume of water, highlighting the importance of reducing fish activity to maintain water quality (Gomes et al. 2003; Portz et al. 2006).

The objective of this study was to determine the efficacy and cost effectiveness of one possible transportation solution by reducing the PCA within transport tanks. To this end, we compared the post transfer survival of the current Unstructured Transport System (UTS) to the alternative, Stratified Transport System (STS), during routine commercial halibut transports.

5.2 Materials and methods

5.2.1 Trial

5.2.2 Fish and rearing conditions

A population of 10,689 commercially-produced Atlantic halibut juveniles (approximate mean weight = 550 g) were held in a single rearing tank under natural photoperiod at a land-based facility in Nova Scotia, Canada. Water was supplied from a saltwater well at a temperature of 7.6°C and salinity of 25 ‰ at the time of transport. Fish were fed a commercial diet and were removed from feed a minimum of 48 h prior to transport. All fish were transferred to a single 7m deep, flat bottomed polar circle sea cage with a circumference of 70 m at a commercial finfish aquaculture site in New Brunswick, Canada (Appendix 1). Water temperatures at the receiving site were 5.9°C with a salinity of 32‰ at the time of transport.

5.2.3 Transport

This trial utilized routine commercial transports that ranged from 12 to 16 h per trip from loading to sea water entry and covering a distance of 380km. Transports took place over three consecutive days from December 16-18, 2008. A single, live haul truck outfitted with five individual 5.0m^3 transport tanks (Dura Tech™, Nova Scotia, Canada) was used to move all three loads. Each transport tank was supplied with an adjustable flow of liquid oxygen distributed via a centrally located fine pore ceramic diffuser. Tank volumes were mixed and aerated by an airlift located in one corner of each tank. Airlifts were supplied by a single gasoline powered blower.

5.2.4 Study design and treatments

Two treatment groups were compared in a controlled trial with treatments replicated in two identical loads. The Unstructured Transport System (UTS) currently used by the industry served as the control for the alternative Stratified Transport System (STS) (Fig. 5.1). In each load, two of five available transport tanks were arbitrarily chosen to receive either a UTS or STS treatment. The halibut were crowded in the rearing tank and dipped into a holding box immediately outside the tank (Fig. 5.2). Study enrolment coincided simultaneously with the loading of the three tanks in each load that were not enrolled in the study, thereby approximating a systematic selection. Halibut were dipped from the holding box and randomly allocated to one of two treatments systematically by the placement of a single jet-injected intradermal alcian blue mark (Thedinga et al. 1997). One worker marked fish cranially, identifying STS fish while the second worker marked fish caudally, identifying UTS fish (Fig. 5.2). Taggers switched mark placement at the halfway point to minimize tagger bias. Ambicoloured fish (Bolker & Hill 2000) were not allocated to transport treatments because alcian blue marks were indistinguishable against the dark pigment and therefore transported untagged. A total of 1146 STS and 1009 UTS fish were tagged. STS tanks received 785fish/tank (431 kg), while UTS tanks received 700 fish/tank (385 kg). The PCA within tanks was estimated using equation (1) described by Reig et al. (2007).

$$PCA = 100 \times \frac{TSA \times N}{A} \quad (1)$$

Where TSA is the average total surface area (cm^2) of each fish, A is the available area (cm^2) for settlement within the transport system and N is the number of fish per transport tank.

and

$$TSA = 11.224 \times W^{0.5716} \quad (2)$$

TSA was calculated using equation (2) which gives the relationship of fish weight (W) in grams to TSA, as developed by (Reig et al. 2007) for California halibut (*Paralichthys californicus*).

The UTS fish were loaded directly into the unstructured transport tanks by dip net, whereas STS cages were loaded with 30-34 fish in each of the 24 coated wire cages (18cm x 61cm x 91cm, with 3.3cm^2 mesh) and stacked four high within transport tanks (Fig. 5.1). Upon arrival to the wharf, fish were offloaded by dipping from the UTS and emptying the dip net into a righting box. In contrast, STS cages were lifted individually from transport tanks then tipped into the righting box. Once placed into the righting box, fish were sluiced into the cage. All handling activities following the placement into the righting box were identical for both treatments. The depth of the sea cage was temporarily shallowed to approximately 1.5m, to facilitate maneuverability between the wharf and the temporary holding location that was approximately 100m from the wharf. Fifteen days following the final load, weather conditions permitted moving the entire cage approximately one kilometre to the finfish site where the cage bottom was dropped to a normal depth of 7m.

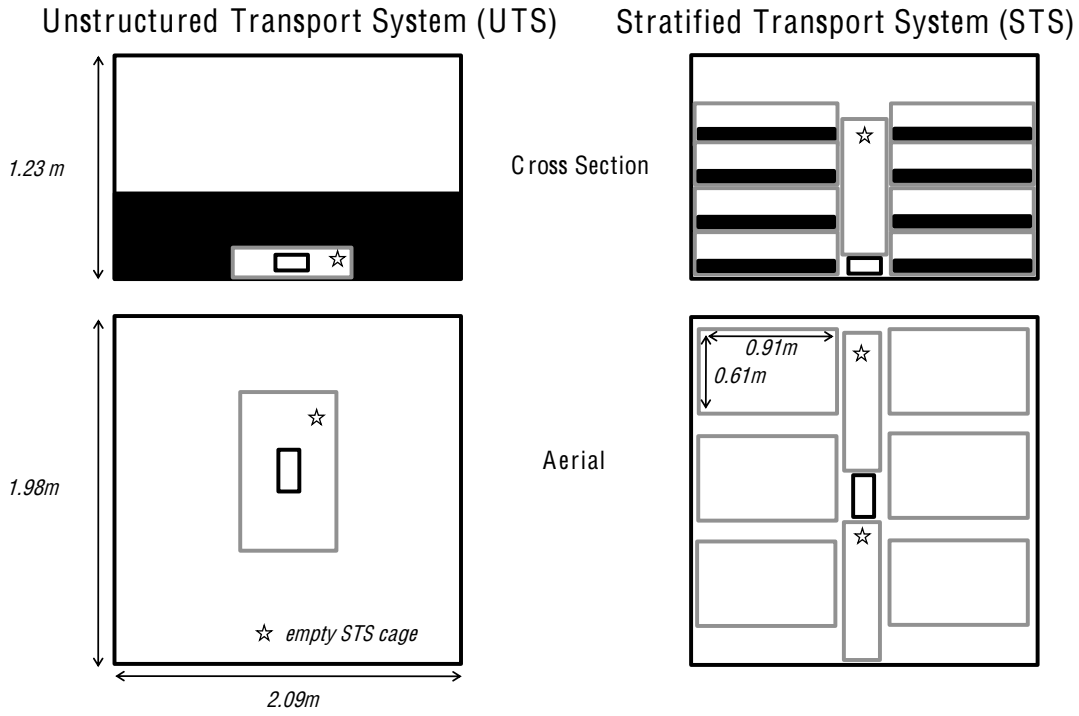

Fig. 5.1. Cross sectional and aerial views of the Stratified (STS) and Unstructured (UTS) Transport Systems. The cross sectional view shows the hypothetical distribution of settled halibut (black shading) in transfer tanks. The aerial view illustrates the layout of the wire cages (gray outlined box) and liquid oxygen diffusers (black outlined box) within transport tanks. Empty wire cages (starred boxes) were used to fill the space between stocked wire cages to prevent shifting during transport. In UTS treatments, the liquid oxygen diffuser was placed inside an empty STS Cage, preventing fish settlement directly on the diffuser.

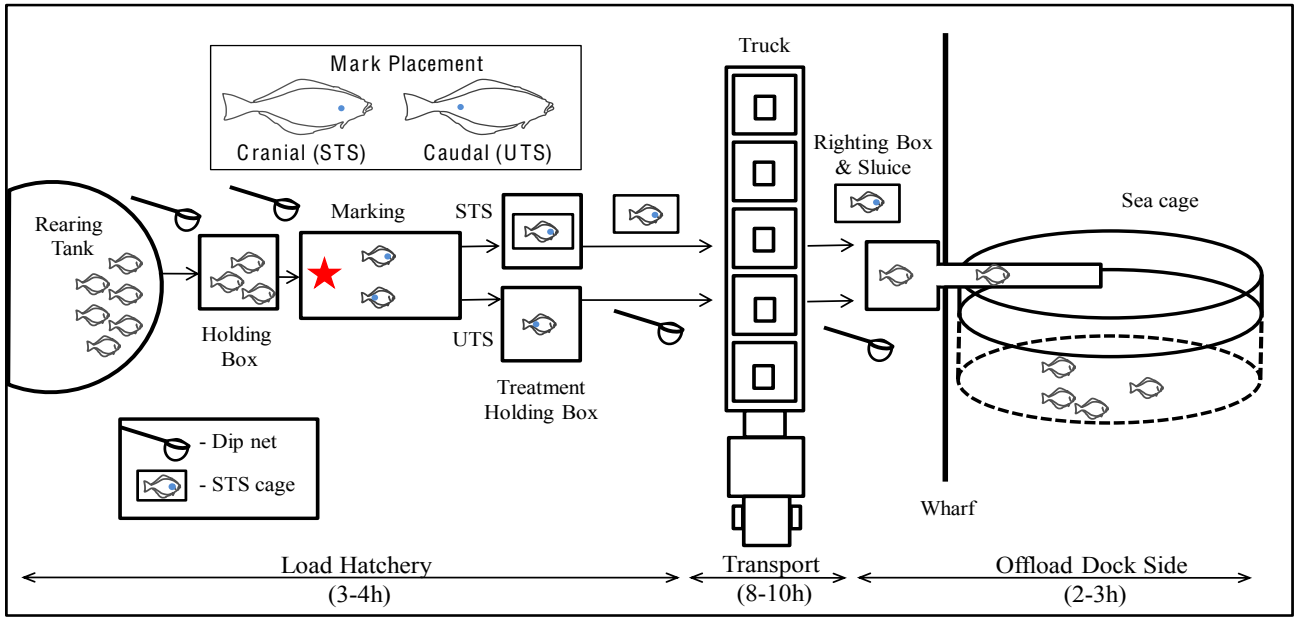

Fig. 5.2. An overview of the handling and transport procedure from the hatchery to the sea cage site. Handling points where fish were netted are indicated by dip nets, the point of randomization to transport treatment is identified by a star.

5.2.5 Water quality and temperature

For both the STS and UTS transport tanks, dissolved oxygen concentrations were monitored every 1.5-2 hrs. using a handheld dissolved oxygen meter (OxyGuard, Birkeroed, Denmark). Water samples were collected from below the water's surface in transport tanks at three distinct time points (prior to loading, temporal mid-point and prior to unloading). Water samples were analyzed to determine pH and salinity using a 6000XLM Multiparameter Water Quality Monitor (YSI, Ohio, USA) at Fisheries and Oceans Canada, Biological Station, St. Andrews, New Brunswick, Canada. Water temperatures within transport tanks were recorded (min^{-1}) using Hobo underwater temperature loggers (Onset, Massachusetts, USA).

5.2.6. Mortality data collection

Commercial divers collected mortalities every 3-4 days, weather permitting. Identification of marks (presence and location) was recorded by farm staff while enumerating mortalities in the 34 days following the final transport. A period of 30 days post-transport was chosen because it has been determined sufficiently long to observe delayed mortality resulting from stress in Pacific halibut (*Hippoglossus stenolepis*) (Davis and Olla, 2001). This is also the same period of time that the salmon aquaculture industry typically uses to evaluate post-transport mortality (Nomura et al. 2009)

5.2.7 Statistical analysis

The effect of UTS and STS treatments on post transport survival was compared using time to event data and analyzed using a non-parametric Kaplan Meier survival analysis with survival functions tested using a log rank test (Dohoo et al. 2003). Cumulative mortality on day 34 was compared using a chi-square test. All statistical analyses were performed using the statistical package STATA 10 (College Station, Texas).

5.2.8 Cost-benefit analysis (CBA)

5.2.8.1 Stochastic modeling

A one-sided stochastic Cost-benefit analysis (CBA) (Drummond et al. 1997) was modeled in MS Excel 2007® using the ModelRisk 3.0 add-in (Vose Software, Colorado, USA). The costs associated with STS adaptation (including: implementation, refinement and maintenance costs) were held constant for the entire analysis whereas, benefits were permitted to vary. Parameters used to compute benefits were sampled from user defined probability distributions (Table 5.1) and the model run 10,000 times using a Monte Carlo simulation. Parameters based on expert opinion were distributed according to a PERT distribution (Vose 2000), where a minimum, most likely, and a maximum value are specified. The proportion of post transport mortality in the STS and UTS treatments were distributed using Beta distributions (Vose 2000) specified by alpha(α) and beta(β) values representing the number of mortalities and survivors, respectively. The benefits associated with the STS were modeled as three separate components:

$$B_{TE} = \left[\left[\frac{At}{Sn_2}\right] - \left[\frac{At}{Sn_1}\right]\right] \times Tc \quad (3)$$

Where B_{TE} is the benefit in transport efficiency by using a STS over a UTS, denoted by the subscripts (1 and 2), respectively. At, is a constant representing the number of halibut juveniles transported annually. Sn, is the number of juveniles that can be stocked and transported in a single load for the respective transport system and Tc is the transport cost of the load.

$$B_M = At \times Jc\ (M_2 - M_1) \quad (4)$$

Where, B_M is the benefit from reduced post-transport mortality of STS compared to UTS. M is the percentage of stock expected to be lost due to post-transport mortality for each transport system and Jc is the cost of a single halibut juvenile.

$$B_R = \left[\left[\frac{At \times M_2}{Sn_2}\right] - \left[\frac{At \times M_1}{Sn_1}\right]\right] \times Tc \quad (5)$$

Where, B_R is a restocking transport benefit acknowledging the reduction in transport costs associated with the delivery replacement fish to replace those lost in the original transport.

$$B_{STS} = \frac{B_{TE} + B_M + B_R}{1 + D^N} \quad (6)$$

A final fourth equation summarizes the Present Value (PV) benefit (B_{STS}) of utilizing a STS over a UTS when benefits are discounted at the rate D, over N years (1, 2, 3, 4, 5). The influence of model inputs were evaluated by a sensitivity analysis concluding the relative importance of each input parameter.

Table 5.1. Parameters used in the stochastic cost-benefit analysis and associated distributions used in the model.

Parameters	Distribution	Values	Data source
Costs			
STS Cages ($)[a]	Fixed	10,224	Rainbow Net and Rigging Ltd.
STS loading system ($)	Fixed	25,000	Author Estimate
Annual maintenance ($·year^{-1})[b]	Fixed	1000	Author Estimate
Benefits			
Mortality STS (M_1)	Beta (α, β)	(128, 1018)	Author Unpublished Results
Mortality UTS (M_2)	Beta (α, β)	(144, 865)	Author Unpublished Results
Stocking Density STS (Sn_1) (n·load^{-1})	PERT(a,b,c)[c]	(3600, 3936, 4000)	Author Unpublished Results
Stocking Density UTS (Sn_2) (n·load^{-1})	Fixed	3500	Scotian Halibut Ltd.
Transport Cost (Tc) ($)[d]	PERT(a,b,c)[c]	(2750, 3000, 3900)	MacIntosh Trucking Ltd.
Juvenile Cost (Jc) ($)	PERT(a,b,c)[c]	(8, 12, 16)	Scotian Halibut Ltd.
Discount Rate (%)	PERT(a,b,c)[c]	(6, 8, 10)	Guy et al., 2009
Assumptions			
Annual Juvenile Delivery (At)	Fixed	50,000	Scotian Halibut Ltd.

[a] Cost to outfit one truck [b] 5-year service life, [c] where (a,b,c) refer to (minimum, most likely, maximum), [d] Cost per load

5.2.8.2 Data sources

The previously mentioned controlled trial provided the differential mortality data for the stratified and unstructured transport systems as well as stocking densities estimates for the STS. Stocking densities for the UTS where attained from current industry practices (Stuart et al. 2010). Transport costs reflect current fuel costs and transport rates of which fuel costs make up approximately 30% of the total cost (Carmen Macintosh, pers. comm.). The minimum and maximum transport cost values were constructed by speculating a 25% drop and a 50% rise in fuel costs, respectively. The cost of halibut juveniles was provided by recent quotations for similar sized halibut juveniles (Scotian Halibut Ltd., pers. comm.) The minimum and maximum values for juvenile cost were speculated by the authors using the following logic: an increase in the size of the halibut farming industry would likely cause juvenile prices to drop due to economies of scale and competition between juvenile producers. Conversely, juvenile prices could rise with the increased production of costlier all-female stocks, which is a currently targeted strategy. The cost of the STS cages was provided by a quotation from a private supply company (Rainbow Net and Rigging Ltd., New Brunswick, Canada). The STS loading system cost and annual maintenance costs are the authors estimates based on equipment required for refinement of the STS, which are discussed later. A most likely discount rate was provided from a recently published commercial aquaculture project (Guy et al. 2009). An annual juvenile delivery of 50,000 was used to reflect the number of juveniles currently produced and delivered to a commercial halibut farmer rearing juveniles to market size in sea cages in Atlantic Canada.

5.3 Results

5.3.1 Transport water quality monitoring

All monitored water quality parameters were within normal limits during all treatment and load combinations. Dissolved oxygen concentration measured at the surface ranged from 11.0-28.0 mg/l during transport with an average of 19.9 mg/l., no hypoxic events were observed. Water temperature within transport tanks ranged from 7.1-7.9°C. There was a 0.5°C cooling of water temperature in all transport tanks over the course of transport due to cooler ambient air temperatures. Salinity was stable within the range of 25.3-25.7‰ and pH in ranged from 6.5-7.7, with water pH highest at the beginning of transport and stabilizing by the mid-point of transport. No appreciable differences in dissolved oxygen, water temperature, salinity or pH were noted between the STS and UTS.

5.3.2 Post-transport mortality

Peak mortality occurred between 9-17 days post-transport with 6.5 and 10.0% cumulative mortality observed during this period for STS and UTS, respectively. Mortality at 34 days post-transport was 11.2 and 14.3% for STS and UTS, respectively (Fig. 5.3.). The overall survivor functions of STS and UTS treatments in the 34 days post-transport were significantly different (P-value =0.028). On day 34 post-transport, mortality was significantly lower by 3.1% in the STS treatment (P-value=0.031). The cumulative mortality of the entire transported population (tagged and untagged fish) was 32.1%.

5.3.3 Transport system stocking

On average, PCA was 237% in the STS compared to 752% in the UTS, while stocking densities in the STS system were on average 12% higher.

5.3.3 Cost-benefit analysis

The stochastic CBA model estimated that 5 years after a $40,224 investment into a STS, an average BCR of 3.12 (95% CI = 2.17-4.12) with a cumulative five year NPV of $85,174 (95% CI, $46,906- $125,630) can be realized. On average, investment in a STS showed positive economic returns two years following investment, with a positive benefit cost ratio (BCR) of 1.31 (95% CI = 0.68-2.00) (Fig. 5.4). The sensitivity analysis identified the difference in post-transport mortality between the STS and UTS as the most critical input influencing the profit improvement of STS adaptation (Fig. 5.5).

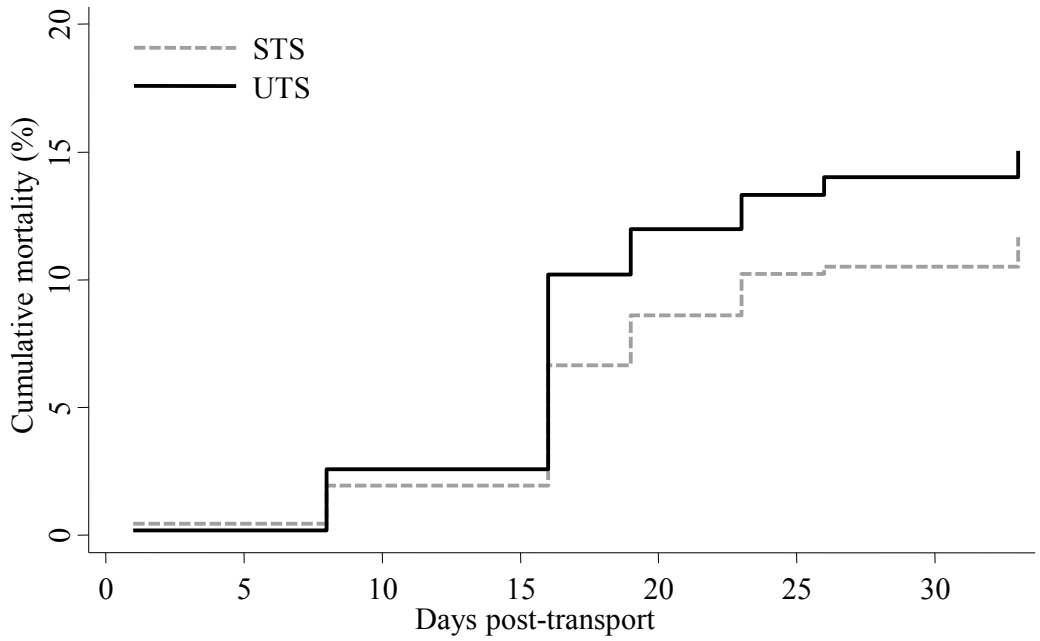

Fig. 5.3. Cumulative post-transport mortality curves for Atlantic halibut juveniles transported by a Stratified Transport System (STS) and an Unstructured Transport System (UTS) in the 34 days post-transport.

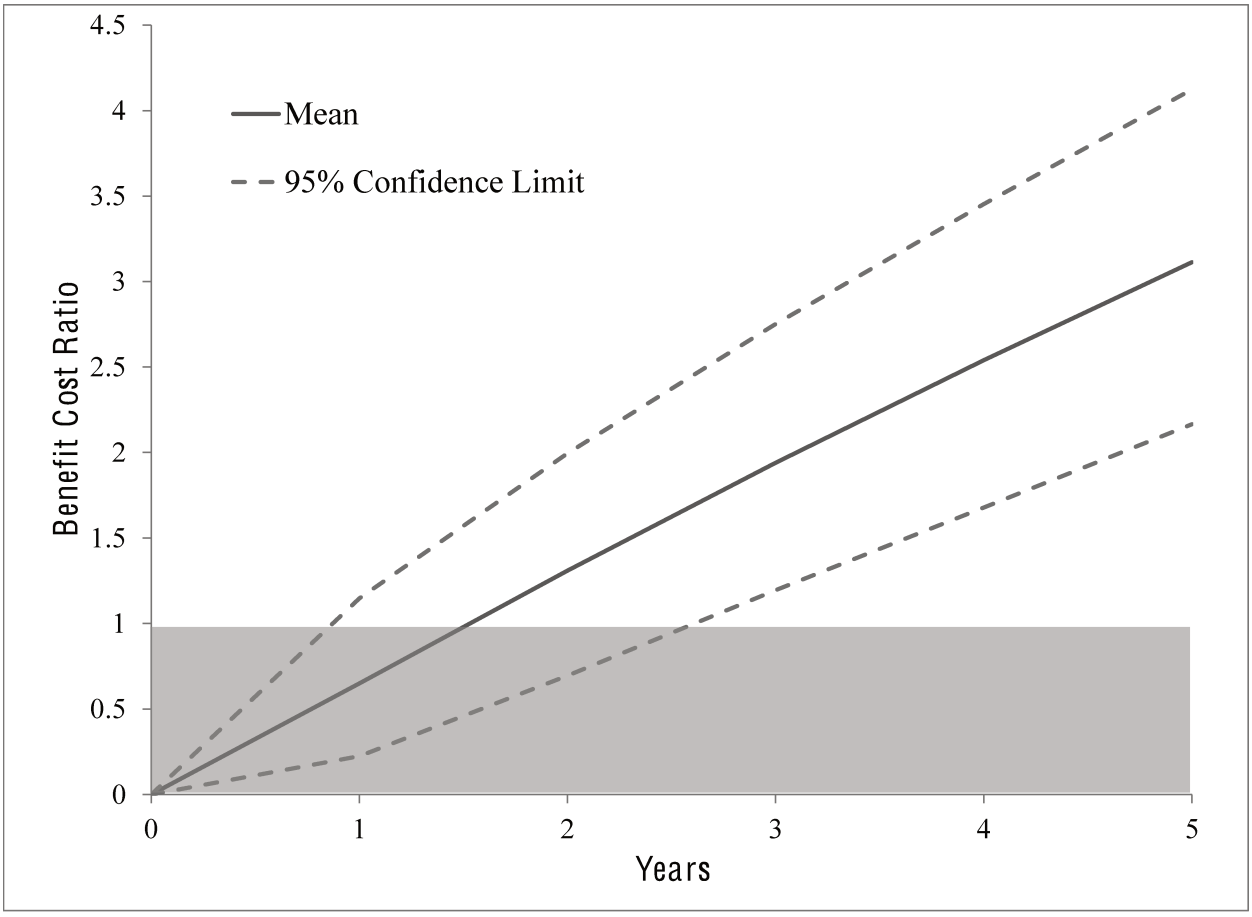

Fig. 5.4. Benefit cost ratio (BCR) of investment into a Stratified Transport System (STS) over a 5 year period. The area above the shade indicates a profitable BCR.

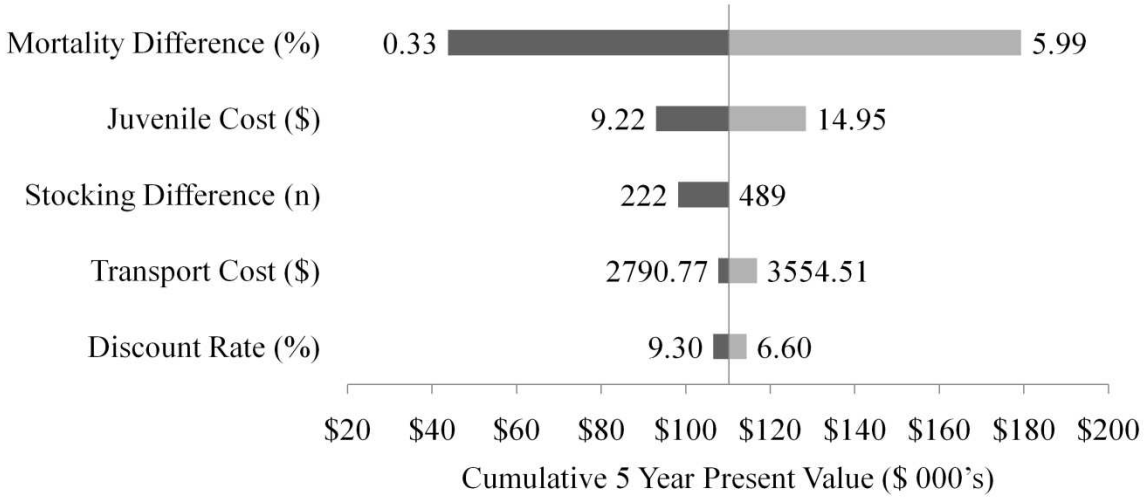

Fig. 5.5. A relative comparison of cost-benefit model factors and their influence on the cumulative 5 year Net Present Value benefit of utilizing a Stratified Transport System (STS) over an Unstructured Transport System (UTS). The numbers embedded in the figure represent the 95% CI for input factors modeled stochastically from theoretical distributions.

5.4 Discussion

5.4.1 Comparison of post-transport mortality

Halibut stocked at 237% PCA in a STS had significantly better survival than those stocked at 752% PCA in the UTS. These results correlate with those of Sulikowski et al.(2006) who demonstrated that cortisol levels of winter flounder (*Pseudopleuronecies americanus)* juveniles transported at densities up to 400 PCA returned to baseline values within 48 hours post-transport, whereas those transported at 600 PCA remained elevated. Although no post-transport mortality was observed in any of their treatments, investigators interpreted the elevated cortisol levels in the 600 PCA treatment to be a biologically significant difference and likely to compromise post-transport survival, upon release into the wild (Sulikowski et al. 2006).

Based on our observations and prior publications, we suggest four potential hypotheses why reducing PCA increases post transport survival of transported halibut. 1) Stratifying the halibut within the tank likely promotes water movement around the fish (Reig et al. 2007) maintaining a homogeneous tank environment and preventing the potential for "dead spots". 2) Stratification may also reduce the frequency and severity of fish-to-fish contact. Mechanical abrasion from fish-to-fish contact is implicated as an important transport related stressor while also compromising the integrity of mucous barriers (Ross and Ross 2008) which are critical to maintaining proper osmoregulation (Eddy 1981) and immune function (Shephard 1994). Anecdotal observations of fish at offloading and of collected mortalities failed to suggest a difference in physical damage to the fish between the two transport systems. However, it is possible that the higher PCA in the UTS resulted in more contact and subsequent damage to the epithelial and mucous barriers, contributing to the higher mortality observed in the UTS. 3) From

a handling standpoint, once the fish are loaded into the STS cages they can be moved as units, removing the need for two "chase and dip" handling points in the transportation process. A reduction in this type of handling can be expected to substantially lower cumulative stress related to transport (Robertson et al. 1988; Iversen et al. 1998). 4) Lastly, and perhaps most importantly, stratifying the fish within the transport system increases settlement area, allowing the halibut to remain in a natural resting position. Allowing the fish to remain in a resting position will reduce their metabolism, preserve water quality (Portz et al. 2006) and prevent exhaustion (Van Ham et al. 2003).

5.4.1.1 Fish marks

Intradermal jet marking was chosen to identify treatment groups due to its rapid, inexpensive application that can be applied without the use of anesthetic. These marks also have the advantage of being non-toxic to the animal and potential consumer, while also having virtually no biological side-effects (Thedinga et al. 1997). Visual inspection of the mortalities showed the blue intradermal marks to be small, but distinct from the white abocular surface of the fish. Some variability in mark darkness was noticed presumably resulting from the suboptimal performance of jet inoculators, due to freezing conditions during tagging.

5.4.1.2 Tank effects

The inability to identify loads within treatment groups prevented the control of "tank effect", making it impossible to determine if unknown spurious factor(s) may have influenced survival in one particular tank during a particular load. Loads within treatments were to be replicated by placing a single mark at different locations on the abocular side, however highly variable pigmentation patterns on the abocular side of the fish limited the ability to diversify

mark location, as a result only a cranial (head) and a caudal (tail) mark were used to provide unambiguous identification. Anesthetic would be required to permit more precise mark placement or the placement of multiple marks, allowing identification of more than two groups. In this situation the administration of anesthetic was time prohibitive due to loading schedules and would have compromised the generalizability of the trial. The measurement of water quality parameters during transport (water quality, temperature, dissolved oxygen and personal observation) did not indicate substantial differences between treatments, making it unlikely that a mechanical failure affected post-transport survival in any of the treatment tanks.

5.4.2 Factors contributing to overall post-transport mortality

Despite the reduced mortality in the STS group, the overall mortality during this fish movement was well above the conventional 3% post-transport mortality (Stuart et al. 2010). Previous transports to this particular grow-out site in the Bay of Fundy have found post-transport mortality to be higher during late fall and winter transports compared to those in the spring and early summer (Skip Wolf, pers. comm.). In this study, peak mortality occurred more than a week following the last transport, confirming that transport was not an acute cause of death but a substantial and necessary factor. Similar delayed mortality patterns have been observed in other species and reported as "hauling loss" (Gomes, et al. 2003). "Hauling loss" is thought to be caused by osmoregulatory failure (Barton et al. 2003), arising from an increase in gill perfusion, a common secondary stress response deemed necessary to meet the increased metabolic needs of stressed fish (Barton and Iwama 1991). The increased perfusion disrupts homeostasis, causing osmoregulation to become a chronic problem (Eddy 1981). A recorded 7‰ increase in salinity from the shipping to the receiving site may have also contributed to the osmoregulatory distress. The seasonality of post-transport mortality is likely attributable to water temperatures at the

receiving site. Although the water temperatures were well within tolerable limits for halibut (Staurnes 2001), the low and declining water temperatures encountered reduce feeding rates with a concomitant decrease in metabolism that makes recovery from transport stressors energetically challenging during the winter months.

5.4.3 Feasibility of use

A STS modification can be implemented with the minimal cost of new STS cages, as demonstrated by this study. The STS cages received minimal design consideration prior to deployment. Once filled, the size and weight (~17 kg) of the STS cages made loading and unloading slightly longer and more cumbersome than that of the UTS. In future designs, it is recommended that cages be built smaller and more ergonomically to accommodate the comfort and safety of workers. Alternatively, larger cages with associated mechanization for loading and unloading could be a viable option. Based on this study, four recommendations are presented to improve the system. 1) A funnel like sluice to transfer fish dipped from the rearing tank to awaiting STS cages would reduce handling and increase the speed of loading, 2) increase the size of the loading hatch on the transport tanks to permit STS cages to be loaded horizontally, 3) alter the materials used in the design of future STS cages to prevent the premature wear and scratching of the internal surfaces of transport tanks and from a bio-security perspective, STS cages create an added surface to disinfect between transports. Choosing smooth, non-absorbing materials, resistant to common aquaculture disinfectants will be important for maintaining the biosecurity of the system, and 4) a surface or mechanism on the righting box to assist with the opening and emptying of STS cages would greatly improve the ergonomics and efficiency of unloading. Overall, it is believed that these equipment modifications will generate savings in

handling time and further reduce handling stressors, because once loaded into STS cages the fish can be moved as units.

5.4.4 Cost-benefit analysis

The STS was found to be a cost effective solution. This was greatly aided by the relatively low capital cost of STS implementation. Permitting input variables to vary stochastically is beneficial in decision making, particularly when data with known variability are provided by controlled trials. The use of sensitivity analysis is also useful for future decision making when costs are likely to have changed from the figures used in the original analysis, this allows users to reasonably estimate how changes to input parameters will impact final outcomes. Beyond the direct economic advantages of utilizing a STS there are additional intrinsic benefits such as improved fish welfare and likely improvements in fish performance post-transport that are likely to further influence investment into transport systems optimized for flatfish.

Atlantic halibut juveniles transported by a STS had significantly lower post-transport mortality than those transported by the traditional UTS. Increasing the homogeneity of the tank environment in combination with reducing fish activity and handling stress are believed to be responsible for the observed reduction in post-transport mortality in the STS. Investment into a STS was found to be cost effective solution for transporting juvenile Atlantic halibut, with the mitigation of hauling losses being the most significant economic factor contributing to the profitability of the system.

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Chapter VI: GENERAL CONCLUSIONS

6.1 Introduction

Aquaculture continues to grow around the world and Atlantic Canada is no exception. Atlantic salmon (*Salmo salar*) remains the focus of Atlantic Canada's production but over time infectious disease, parasitic infestations, and price fluctuations resulting from international competition have led to disruptions in productivity. These disturbances have resulted in industry-level changes, namely vertical integration and company mergers to reach economies of scale. Diversification of the industry into other species has been considered a solution to protect the industry against fish health and market challenges. A high-market-price, firm, mild tasting white flesh, and limited seasonal availability from commercial fisheries make Atlantic halibut a superior candidate species for aquaculture diversification. However, despite a reliable supply of juveniles in the region, a commercial industry has yet to attract the investment required to diversify the finfish farming sector in Atlantic Canada.

Although substantive knowledge and experience exist for land-based culture of Atlantic halibut, improvement is necessary for further development of efficient cage-culture techniques. Fish health is the main factor that affects the productivity and profitability of aquaculture farms. The inability to mitigate pathogen exposure is a general disadvantage of cage-culture. The presence of only a pilot scale industry, with a limited history of production and only limited treatment interventions for potential fish health challenges, results in reluctant investors.

The objective of this thesis was to provide insight into the productivity of Atlantic halibut in cage-culture conditions in the Bay of Fundy and to identify potential health risks. Toward this

objective, four specific projects were undertaken: i) establishing the impact of developmental malformations on productivity, ii) determining the side-effects of prophylactic vaccination and the optimal location for vaccine injection, iii) validating an external identification tag to facilitate the monitoring of individual Atlantic halibut in randomized controlled trials (RCTs), and iv) designing and testing a novel transport system specific to flatfish, while determining its cost-effectiveness.

6.2 Developmental malformations and sex

In commercial aquaculture, malformations are often ignored or eliminated through culling and are considered a routine cost of production. Studies to determine the prevalence of abnormalities in commercial populations and their impact on productivity are rarely mentioned in published literature (Leatherland & Woo 1999). A better understanding of the prevalence and impact of developmental malformations on productivity allows producers of juveniles to prioritize their efforts on correcting the malformations with the greatest overall impact on production.

Female halibut grew faster and more efficiently than males, an observation previously reported for commercial production (Bjornsson 1995; Power 2009). Male and female halibut had comparable growth until the second sea winter at which time the majority of male halibut became mature with fully developed gonads. Diverting energy from somatic growth to gonad development greatly reduces the overall growth efficiency and results in substantial size differences between male and female fish following the second sea winter and observed at harvest. The use of all-female populations is a potential solution. Scotian Halibut Ltd., the only supplier of halibut juveniles in Atlantic Canada are capable of producing all-female populations

using sex-reversed broodstock. Recently, commercial growers using all-female stocks have found considerable growth variability amongst fish, which they attributed to poor crosses of parental stock (Gerald Johnson, Pers. Comm.). Although all-female stocks are an option for halibut growers, the increased cost of all-female stock should be justified in an economic study prior to adopting this strategy.

The prolonged grow-out period needed to bring halibut to market size requires improved growth performance of culture stock. Culling poorly performing, malformed juveniles prior to sea cage entry was identified as a strategy to increase performance (Chapter II). Incomplete eye migration and cataracts were found to have a negative impact on the growth of affected individuals. By classifying eye migration using a four point scale (Gara et al. 1998) and quantifying the impact on growth performance, culling of these malformations can be optimized. Halibut with moderate and severe incomplete eye migration had significantly decreased growth. Culling of these fish was recommended over culling of all halibut with incomplete eye migration because juveniles with mild incomplete eye migration (7% in this study population) had acceptable productivity, thus avoiding unnecessary culling.

Screening juveniles for developmental malformations prior to purchase is advisable for improving grow-out performance, allowing producers to adjust juvenile prices based on the prevalence and severity of malformations related to reduced growth performance and marketing issues. Quantifying the production impact of developmental malformations informs evidence-based management, potentially reducing the grow-out period by better predicting growth performance using optimally selected juveniles.

6.3 Vaccine side-effects

Although vaccination has become a standard protocol in the production of many aquaculture species, this is not the case with Atlantic halibut. In the sea cage environment, vaccination reduces the need for chemotherapeutics and decreases the frequency of disease outbreaks amongst individuals and populations (Lillehaug et al. 2003; Grave et al. 2008). Disease in production settings can be unpredictable, thereby increasing the need for vaccination while making it difficult to assess its efficacy.

The cost of vaccination is minimal when compared to the cost of disease. For this reason producers are likely to vaccinate fish to mitigate disease risk, despite unproven efficacy of the vaccine, unless vaccines are found to have adverse-side effects. Although the study population monitored (see Chapter III) did not experience the disease exposure required to assess vaccine efficacy, it provided an ideal opportunity to study the potential side-effects of vaccination, thereby providing insight into the considerations for on-farm vaccination practices.

The growth of vaccinated fish was compromised in the six-month period immediately following vaccination, which is a common occurrence in many species (Midtlyng 1997; Midtlyng & Lillehaug, 1998; Pylkko et al. 2000; Gudmundsdottir et al. 2003) likely due to intraperitoneal inflammation resulting in reduced feeding (Midtlyng 1997). This growth disparity was eliminated in later periods with no significant differences observed amongst the vaccination treatment groups and control groups at the time of harvest. The similarity in mortality patterns between vaccinated halibut and unvaccinated control halibut confirmed the lack of disease exposure during the trial, while also demonstrating the safety of vaccination.

Two potential vaccine injection locations were identified and tested. No significant differences in growth or survival were observed between the two vaccination locations. This suggests that efficiency and/or vaccinator preference could dictate the location chosen.

This study was not able to assess protection against disease challenge. This study population was intensely monitored over a four-year grow-out, during which time no infectious disease was observed in the population. With the exception of a minor settlement of sea lice (*Caligus elongatus*), no health concerns were identified.

6.4 External identification

External tags have been widely used to study flatfish in fisheries and aquaculture for decades with little knowledge of their biological impacts on fish. Cost is the usual justification for tag selection rather than evidence-based decisions or what is best for the purpose of study. It has been suggested that, instead of evaluating the cost per tag, investigators should choose tags based on the cost-per-unit of valid data (Bergman et al. 1992). FT4 lock-on tags offer many advantages over Passive Integrated Transponder (PIT) tags such as external visibility, easy removal, and lower cost. However, factors such as tag retention and biological impacts on growth and survival were previously unquantified. By observing a large number of fish in commercial settings this work has established the biological impacts of Floy tags on Atlantic halibut; information that is potentially applicable to other flatfish species. FT4-lock-on tags were found to reduce growth over the entire grow-out period, making them less than ideal for identifying fish in growth studies. On the other hand, high retention rates over relatively long periods are an advantage for long term identification, as compared to other external tags. Although there was some indication that stressful and hypoxic environments may present

challenges for FT4 tagged halibut, FT4 lock-on tags were not observed to affect survival under normal cage-culture conditions. Overall, these results quantified the biases introduced by FT4 lock-on tags, providing evidence of the inadvertent contributions potentially made to growth monitoring studies using external operculum tags.

6.5 Flatfish transport

The unique anatomy and behaviour of Atlantic halibut results in production inefficiencies (i.e., post-transport mortality and low stocking densities) when using equipment designed for Atlantic salmon transport. An effective low cost modification to the conventional unstructured transport system (UTS) used for salmon was designed and tested against currently practiced methods. Atlantic halibut juveniles transported by the stratified transport system (STS) had significantly lower post-transport mortality than those transported by the traditional UTS. Increased homogeneity of the tank environment in combination with reduced fish activity and handling are believed to be responsible for the observed reduction in post-transport mortality in the STS. Investment in a STS was found to be a cost-effective solution for transporting juvenile Atlantic halibut, with the mitigation of hauling losses being the most significant economic factor in the system's profitability.

Transport imposes many unavoidable stressors on fish. Although, the stress of transport is acute, multiple handling events can have cumulative effects and eventually overwhelm the fish, resulting in mortality. Minimizing costly post-transport mortality by utilizing transport equipment that accommodates the biological differences between Atlantic halibut and Atlantic salmon is crucial for the economic performance of the halibut aquaculture industry.

In similar trials, physiological measures of stress hormones are commonly used as study outcomes (Sulikowski et al. 2006). However, these measures represent distinct points in time and make it difficult to interpret meaningful biological or economical differences.

6.6 Randomized controlled trials

Admittedly, diseases are not distributed evenly across all species or environments, but within the aquaculture sector, it is estimated that financial losses due to disease exceed 25% of global production (OIE 2012). As the aquaculture industry continues to grow, so will the threat of disease, emphasizing the need for RCTs to provide evidence regarding the efficacy of treatments and preventive management.

6.6.1 Scale

The research undertaken in this thesis occurred at commercial scales, which has both advantages and disadvantages. Working at or near commercial levels of production provides understanding of problems that are largely misunderstood or completely unknown. Individual fish-level data provide unique perspective on production performance, from which inefficiencies can be identified and solutions proposed.

Conducting commercial scale trials has less direct experimental control. Researchers must accommodate production processes which can limit scientific objectives and at times compromise study findings. Production schedules can change at a moment's notice based on weather forecasts, equipment availability and unforeseen circumstances. Scientific protocols must be adapted to the situation, sometimes requiring compromise and constant attention to the scientific rigour.

Conducting commercial scale trials requires the handling of large numbers of fish in time-constrained and unpredictable environments where variables such as weather, equipment failures, and tides can affect data collection and potentially result in missing data. Missing data are noticeable in longitudinal studies over long periods of time. In addition to missing data, recall difficulties and inconsistencies in data collection over long periods of time can also provide difficulties during data analysis. A small amount of missing data at each sampling point can compound over the study, resulting in relatively few observations with complete data. Analytical methods are available to accommodate missing data; however, in this trial the problem results from missing predictors at specific sampling periods that result in only a fraction of the data being available for analysis. Long-term commercial-scale trials require flexibility to accommodate the unforeseen events that will inevitably occur. The mortality event that occurred on day 189 of the trial (discussed in Chapter IV) is a good example of how unpredictable occurrences can have negative consequences on study objectives.

Producers deal with suppliers, finances, weather, processors, wholesalers and customers, while researchers accommodate funding sources, producer concerns, weather, university expectations, and publication reviewers. Although they have the same long-term goals of making good production management decisions, the immediate objectives of science and farm management can be quite different and may require different decision processes for daily activities.

6.6.2 Improvements to the methodology

Research and development challenges abound when commercializing an alternative aquaculture species. A study population, although costly and time consuming, generates a large

amount of robust data and enables hypothesis generation for future studies. However, the collection of individual fish-level data in commercial environments is labour intensive. One disadvantage of a commercial scale RCTs using individually identified fish is the sheer volume of sampling required at multiple times throughout the production cycle. A sampling event requires a minimum of 12 people and long days with efficiency only attainable through a high degree of organization and training. This type of trial does not provide the opportunity to repeat data collection events. Due to the expense of setting up such rigorous trials, it is efficient to attempt multiple, simultaneous objectives using a single study population. Although cost-effective, multiple objectives have the potential to compromise each other.

Paper records are slow, error-prone and require time-consuming data entry. This study utilized electronic data collection by scanning the tag identification numbers directly into a spreadsheet followed by verbal communication of the remaining measurements (weight, length, physical assessments) to a data recorder entering the data into a weather-proof field computer. This requires audible communication between individuals in noisy environments, potentially contributing to data errors. These events take in excess of 12 hours in field conditions, requiring a high degree of focus and coordination among the study personnel in order to facilitate the required throughput.

The longer the fish are crowded in the seine, the greater the risk of post-handling mortality (Burnley 2011). This was the cause of an unfortunate handling event on day 189 of our study, reducing our halibut population by approximately 50%. When sampled, a fish will typically change hands four to five times, which wears on the mucus layers, introduces dropping potential and delays the return to the oxygen-providing water. Minimizing the number of

handling steps and people required would be advantageous to improve the speed and accuracy of data collection while simultaneously improving fish welfare.

One way to address this challenge is to modify sampling in such a way as to remove the requirement for verbal communication so that a single person can sample and record data. A prototype handling table was designed to accomplish this task (Fig. 6.1). The advantages of this equipment would be a reduction in typographical and communication errors, increased throughput, reduction in personnel required and reductions in handling stress to the fish. An integrated PIT scanner would create a numbered observation within a spreadsheet. The fish would be transferred onto an integrated scale that automatically inputs the weight for that observation. By the touch of the screen, a digitizing board records continuous variables (i.e., fork length) and categorical observations (i.e., incomplete eye migration) can be measured and recorded in the electronic spreadsheet. All data would be recorded and viewable using a tablet screen. This would remove communication errors and permit the data collector to confirm and review data as they are entered. Overall sampling time, effort required and most importantly, fish stress would be reduced by the addition of a fish handling table.

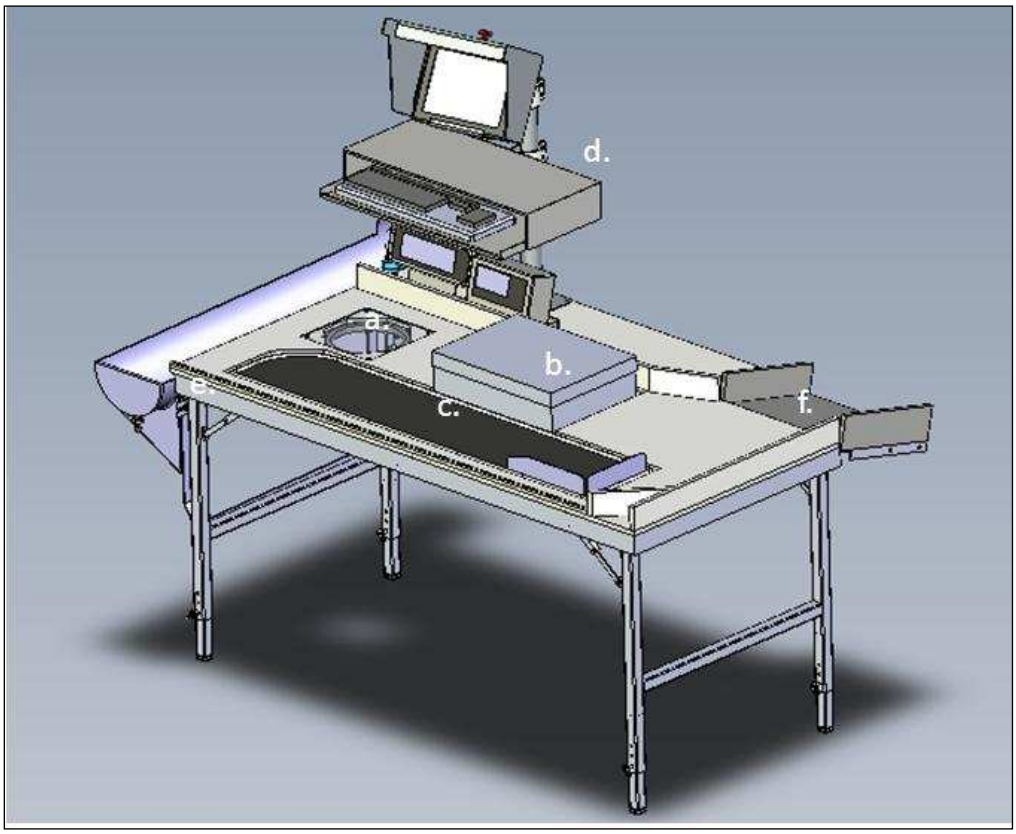

Fig. 6.1. An engineered drawing of fish sampling table prototype designed to collect individual fish level data. The table incorporates a PIT reader (a.), weigh scale (b.), digitizing board (c.) to record categorical predictors and fish length, a water proof tablet computer (d.), inlet (e.) and outlet (f.) sluices to move fish on and off the table.

6.7 Halibut culture going forward

Variability in growth is one of the main challenges to successful cage-culture production observed by this trial. As previously discussed, the goal is to produce 3-5 kg fish for harvest following a 36-month grow-out, but the majority of fish take much longer (Fig. 6.2) with some individuals never achieving this target. The variability in size causes problems executing harvests, requires extra handling and grading of fish prior to harvest, and the smaller fish must be held over or sold at reduced prices. Increasing the growth performance will be important in shortening the production cycle. Avoiding early maturing male fish and malformed fish, shown to reduce growth performance, is a potential strategy to shorten production cycles.

Juveniles remain a significant cost to Atlantic halibut production. This high initial cost is compounded by the sheer length of time required to bring halibut to a reasonable harvest size, with upfront juvenile costs financed throughout the production cycle and resulting in increased cost and financial risk to farmers.

Until growth variability and performance can be improved through domestication, genetic improvement and/or the adoption of all-female populations, the development of a profitable Atlantic halibut aquaculture industry will stall.

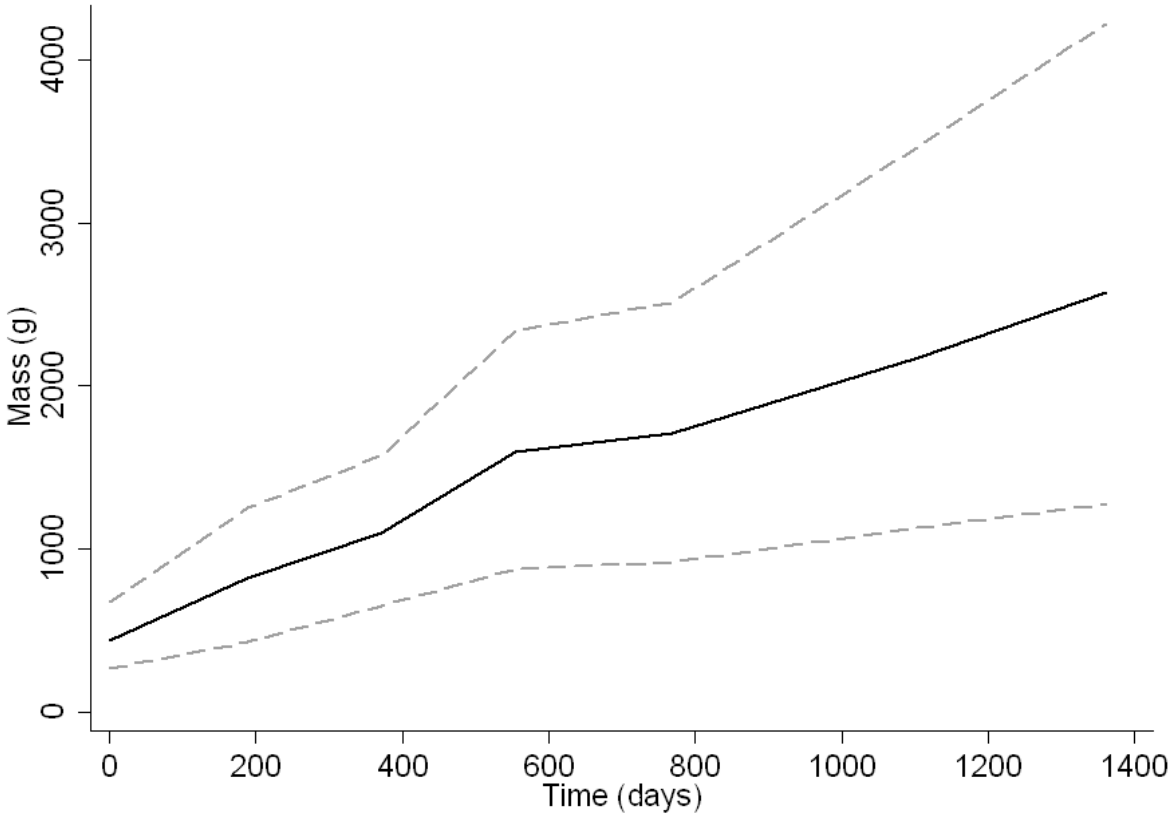

Fig. 6.2. The average mass (g) (solid black) and top and bottom fifth percentiles (dashed grey), showing the variability in mass within the study population of Atlantic halibut during this study.

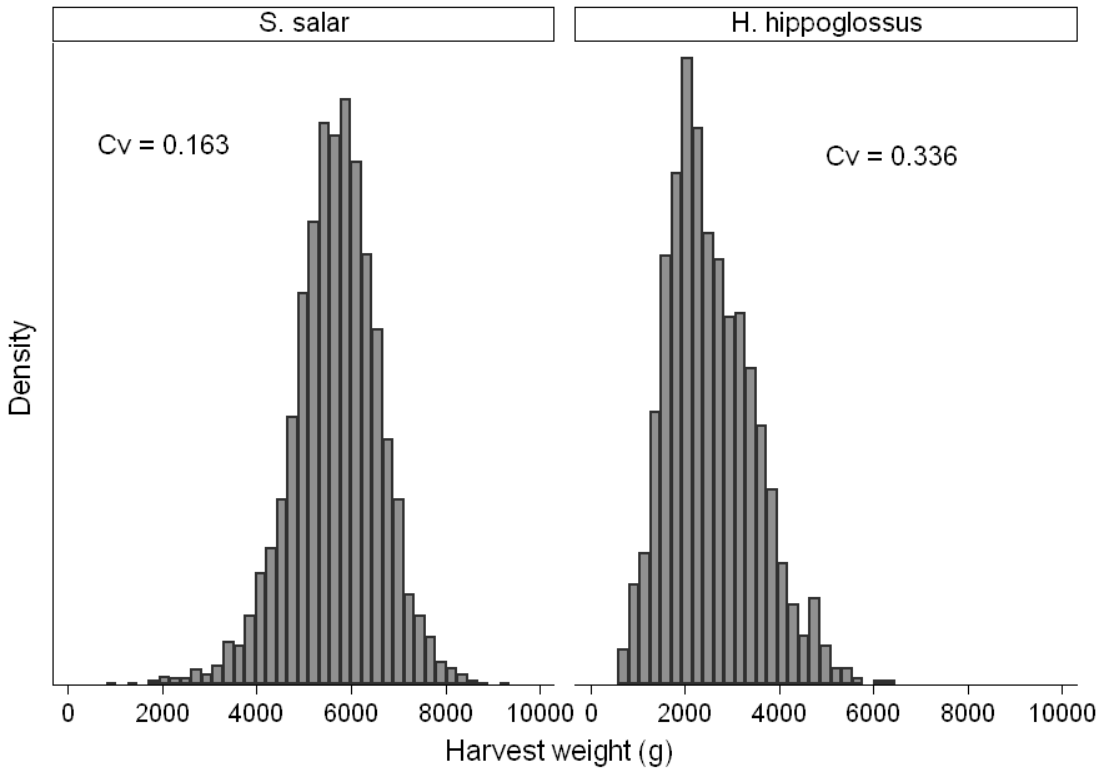

Fig. 6.3. A side-by-side comparison of harvest weights from study populations of Atlantic salmon (*S. salar* data from Burnley 2011) and Atlantic halibut (*H. hippoglossus*; data from this study) grown in a similar area in the Bay of Fundy. The sea cage grow-out period (vaccination to harvest) was 693 days with a vaccination weight of 80g for salmon and an average of 1361 days with a vaccination weight of 441g for halibut. The coefficient of variation (Cv) measures the dispersion of harvest weights in the two study populations.

The need for health interventions is clear, but halibut production lacks a substantial production history and information on the potential of health interventions that are available to producers. Currently, there are no vaccines, antibiotics or other treatments labeled for use in Atlantic halibut and thus dosages, instructions and withdrawal times are unavailable. This leaves responsibility and decisions in the hands of veterinarians when managing health concerns on the farm.

Overall, this thesis provides methods and tools to improve health management and advance the economic understanding of Atlantic halibut production in Atlantic Canada. Although growth performance is still suboptimal when compared to Atlantic salmon production (Fig. 6.3), Atlantic halibut remains a strong candidate for aquaculture diversification in Atlantic Canada, provided productivity and health challenges can be addressed.

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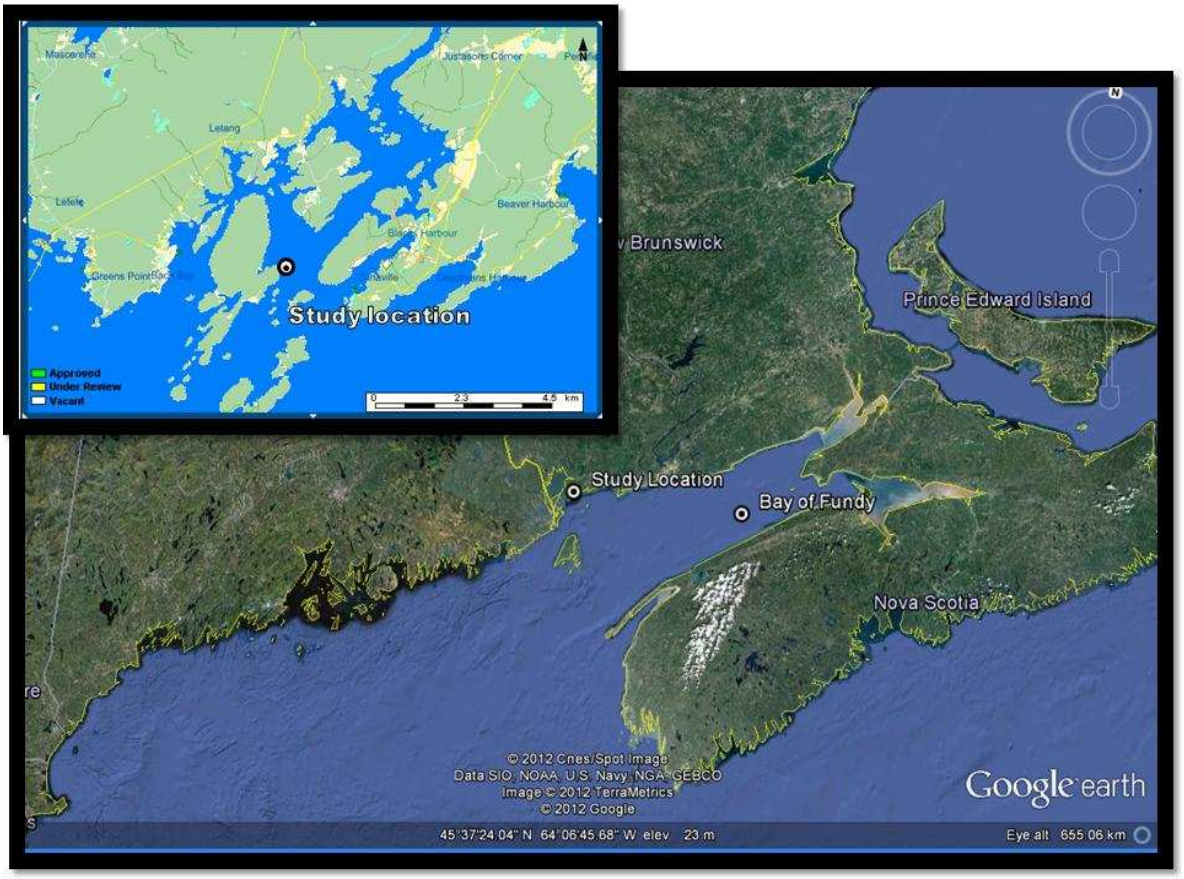
APPENDIX 1

The location of the study population at a commercial Atlantic halibut cage-culture site in Lime Kiln Bay, near St. George, New Brunswick, in the southwest Bay of Fundy.



APPENDIX 2

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APPENDIX 3

The comparison of three predictors used to indicate the level of incomplete eye migration in cultured Atlantic halibut using mixed-models with random effects estimated for each individual fish.

On day 189 of the study a handling event resulted in the mortality of 2712 fish. As a result these fish were unavailable for incomplete eye migration reassessment using a four-point scale on day 372 of the study. These resulted in a large number of fish with missing data for the eye migration predictor causing them to be dropped from the analysis. The data were analysed using mixed-models with random effects estimated for each individual fish. The random effect estimated for each fish influences the analysis over the entire study period. For this reason it was important to ensure that the entire study population was represented in the analysis. Additionally, the handing event resulted in the mortality of smaller (and perceived weaker) fish. The sub-population of fish that survived the handling event was unlikely to be to be representative of the initial population because of differential mortality. Limiting the analysis to fish that were available for incomplete eye migration reassessment could potentially bias the results, particularly when the objective of the study was to estimate the impact of abnormalities on productivity. To address this concern a modelled predictor of incomplete eye migration was constructed. The results from three different incomplete eye migration predictors (Table 1.) were compared. A sensitivity analysis was conducted on the modelled predictor to determine the potential impact of misclassification.

Table 1. Examples of the incomplete eye migration predictors compared and how they were constructed.

	Dichotomus Migration (T 0)	Migration Re-asessed	Incomplete Eye Migration Predictors Dummy variables				n
Model A			None	Mild	Moderate	Severe	
	0	1	0	0	0	0	3956
	1	0	-	-	-	-	
	1	1	0	0	1	0	
	0	0	1	0	0	0	
Model B			Absent	Present			
	0	-	1	0			4560
	1	-	0	1			
Model C	1	1	0	0	1	0	
	0	1	1	0	0	0	4560
	1	0	0	0.505	0.275	0.22	
	0	1	0	1	0	0	
Model C - Sensitivity (All Mild)			None	Mild	Moderate	Severe	
	0	1	0	0	0	0	4560
	1	0	0	1	0	0	
	1	1	0	1	0	0	
Model C Sensitivity (All Severe)			Absent	Mild	Moderate	Severe	
	0	1	0	0	0	0	4560
	1	0	0	0	0	1	
	1	1	0	0	1	0	

Grey shadow indicates manipulated data (-) = missing data

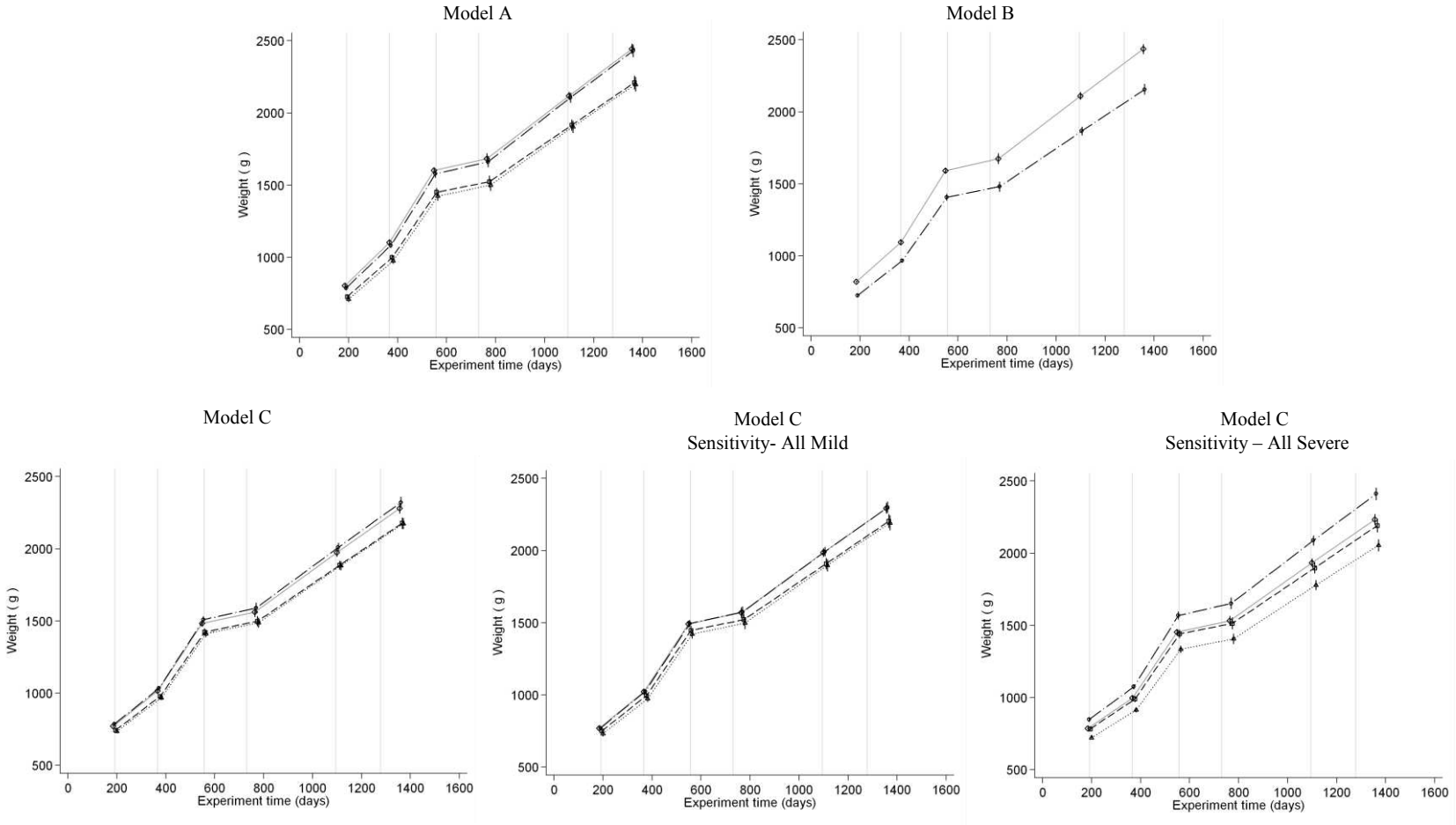
Analysing real data (model A) as collected requires little explanation, as there are no assumptions about the validity of the predictor. The concern of this approach is that the analysis is conducted on a subset of the population, and we do not fully understand the mechanism that defined the sub-population. Given the conditions under which this sub-population was formed it is likely to have differential growth from the initial study population. Model A analyses 3956 individual fish with a minimum, average and maximum of 1, 2.6 and 6 observations, respectively.

Model B uses the dichotomous predictor assessed at the beginning of the trial which includes the observations generated by 4560 individual fish with a minimum of 1 observation, a maximum of 6 observations and an average of 2.4 observations per fish. Similar to model A, this model analyses the data as-collected. However, the dichotomous predictor has limitations. Understanding the impact of incomplete eye migration severity on productivity is important for developing quality criterion and culling practices for halibut juveniles in commercial farm settings. Limiting the interpretation of the results to a dichotomous predictor leaves little opportunity to develop efficient management practices.

Model C uses all the data available, this includes the observations generated by 4560 individual fish with a minimum of 1 observation, a maximum of 6 observations and an average of 2.4 observations per fish. These fish were assigned a predictor based from their dichotomous incomplete eye migration status assessed at the beginning of the trial and a weighted average score based on the distribution of incomplete eye migration severity observed in the remaining

population which was calculated as 50.5% mild, 22.5% moderate and 22.0% severe. A sensitivity analysis compared results of the modelled predictor to the results of the two most extreme possible assumptions: all fish unavailable for reassessment classified as mild or severe . Using a modeled predictor enabled the estimation of random effects for each fish in the study population, and therefor the results of this analysis are not biased to only those that have survived.

The in the end the choice of model does little to change the overall interpretation of the results. The sensitivity analysis demonstrated how misclassification of the eye migration severity could potentially impact the results. When the missing fish were assigned to a particular group (mild/severe) it is evident that the average weight of that particular group was reduced over the entire study period. This suggests that the fish that died during the handling event were smaller fish. Analysing only the real data, restricts the interpretation of the results to the fish that survived the handling event, which are no longer representative of the entire population. Considering that the final interpretation of the results for Model A and Model C are similar, Model C was determined to be the most appropriate method to analyse the data.



The comparison of results generated from three different predictors of incomplete eye migration and a sensitivity analysis of the modelled predictor. Each plot shows the mean weight profiles of Atlantic halibut modelled using three different predictors of incomplete eye migration. Normal eye migration (solid line) and incomplete eye migration: mild (long-dash), moderate (dash) and severe (dot).Values are mean (± 95% CI). All groups were measured on the same day, but data have been offset for clarity.

APPENDIX 4

Vaccine package inserts for vaccines used in the trial.

SAFETY INFORMATION

***Aeromonas salmonicida, Vibrio anguillarum-ordalii-salmonicida* Bacterin**

LIPOGEN FORTE **W-11-2**

TRANSPORT REGULATIONS	Avoid exposure of the goods to temperatures in excess of 30°C (86°F). Do Not Freeze.
MANUFACTURER	Aqua Health Ltd, 37 McCarville St.,West Royalty Industrial Park, Prince Edward Island, Canada, C1E 2A7.
RECOMMENDED USE	As an aid in the prevention of furunculosis, vibriosis and cold water vibriosis.
PACKAGING	1000 mL or 750 mL flexible plastic intravenous-type bags with clamp off seal and screw top cap.
METHOD OF USE	Injection - 0.1 mL intraperitoneally
APPROVAL	USDA Vet. Permit No. 335 Product Code: 2138.01 Can Vet Biol Est No. 28 Product Lic. No: 870BA/A16.0/A8
HAZARDOUS INGREDIENTS	Bacterial endotoxin; formalin, emulsifiers Adjuvant: pharmaceutical grade oil;
RISKS	Low risk from ingestion or eye contact; moderate risk from injection
SAFETY PRECAUTIONS	Avoid ingestion or eye contact. If product is accidentally injected, take precautions against localised sepsis and report the incident immediately to the safety officer and **seek medical attention without delay.** Anaphylaxis (shock) may occur in individuals hyper-sensitive to gram-negative bacteria following accidental injection of product. Prior to use of the product, operators should seek medical advice relating to the recognition and immediate treatment in the case of anaphylaxis. Epinephrine or an equivalent drug should be available for immediate use following these instances.

a NOVARTIS company

DISPOSAL	Use the entire contents of bag when opened. Dispose of surplus vaccine by incineration.
STORAGE	Store at 2-7°C (33-36°F); DO NOT FREEZE
ACTION IN CASE OF SPILLAGE	Mop and wash the area of the spill, dispose of product in accordance with local waste disposal regulations.
FLAMMABILITY	Low risk, high ignition temperature.
HARMFUL EFFECTS FIRST AID	EYES: Irritant/irrigate thoroughly with water. INJECTION: Mild to moderate reaction to bacterial endotoxin or the adjuvant, if unusual swelling or redness occurs **SEEK MEDICAL ATTENTION IMMEDIATELY**. INHALATION: None Known. SKIN: None known/wash affected area.
ADDITIONAL INFORMATION	Product is a formalin inactivated, whole-cell bacterin containing no live organisms.
FOR FURTHER INFORMATION CONTACT	Aqua Health Ltd. 37 McCarville St. Charlottetown, P.E.I. Tel: (902)566-4966 Fax: (902)566-3573
DATE	September 22, 2004

ALPHA Ject® 4000

Aeromonas Salmonicida-Vibrio Anguillarum-Salmonicida Bacterin

Package Insert

To the physician:
This product contains mineral oil. Even if small amounts have been injected, accidental injection with this product can cause intense swelling, which may, for example, result in ischaemic necrosis and even the loss of a digit. Expert, PROMPT, surgical attention is required and may necessitate early incision and irrigation of the injected area, especially where there is involvement of finger pulp or tendon.

Repeated self injections may aggravate the effects or cause anaphylactic shock.

PRECAUTIONS:
Store at 2-7 °C (35-45 °F). Do not freeze.
Use the entire contents when first opened.

PRESENTATION:
ALPHA JECT 4000 is supplied in 500 mL (5000 dose) UVO injection bags.

MANUFACTURED BY:
PHARMAQ AS, Overhalla, Norway

DISTRIBUTED BY:
Syndel Laboratories Ltd., Qualicum Beach, BC

CA-4-3006-0.5 IN

PHARMAQ AS
Harbitzalleén 5
P.O.Box 267, Skøyen
N-0213 Oslo
Norway

PHARMAQ

ALPHA Ject® 4000

Aeromonas Salmonicida-Vibrio Anguillarum-Salmonicida Bacterin

Package Insert

ALPHA Ject® 4000

Aeromonas Salmonicida-Vibrio Anguillarum-Salmonicida Bacterin

Package insert

ALPHA Ject® 4000

Aeromonas Salmonicida-Vibrio Anguillarum-Salmonicida Bacterin

Package insert

ALPHA Ject® 4000

Aeromonas Salmonicida-Vibrio Anguillarum-Salmonicida Bacterin

Package insert

For Veterinary Use Only

DESCRIPTION:
A furunculosis, vibriosis and cold water vibriosis vaccine containing: formalin inactivated cultures of *Aeromonas salmonicida* subsp. *salmonicida*, *Vibrio anguillarum* serotypes O1 and O2 and *Vibrio salmonicida*.

INDICATIONS:
ALPHA JECT 4000 is recommended for use as an aid in the prevention of furunculosis caused by *Aeromonas salmonicida*, vibriosis caused by *Vibrio anguillarum* serotypes O1 and O2, and cold water vibriosis caused by *Vibrio salmonicida* in healthy Atlantic salmon, 15 g or larger.

DIRECTIONS FOR USE:
Bring slowly to 15-20°C and shake well before use. Vaccinate healthy fish, 15 g or larger, intraperitoneally with 0.1 mL of bacterin per fish. Diseased fish should not be vaccinated. It is recommended that the fish be anaesthetized prior to vaccination. Deposit the entire dose into the abdominal cavity in order to reduce the risk of side effects. The injection needle should have an appropriate length to penetrate the abdominal wall by 1-2mm. The entire needle should be inserted into the midline about 1-1.5 pelvic fin lengths anterior to the base of the pelvic fin.

CAUTIONS:
1. Use only at water temperatures above 1°C.
2. A period of 400 degree days following the vaccination is recommended before exposure to *Aeromonas salmonicida*.
3. Only administer if the vaccine appears as a homogeneous, cream coloured emulsion after shaking. If the vaccine shows sign of a brownish water phase in the bottom of the container, the vaccine should not be used for vaccination. Contact the distributor for further advice.
4. Ensure that all vaccination equipment is sanitized before use.
5. Dispose of any unused product according to local bio-medical waste disposal requirements.
6. Store out of the reach of children.
7. Vaccinate healthy fish only. Fish incubating disease or stressed due to shipping, malnutrition or parasitism may not achieve an adequate immune response.
8. Do not use in fish intended for breeding.

CAUTIONS (CONTINUED):
9. Side effects in the form of visceral adhesions and pigmentation may be observed. A transient growth rate reduction due to the stress of handling may be noted following vaccination.
10. Some antibiotics may influence the immune response in fish, thorough consideration should be given regarding the use of antibiotics during the immunization period.
11. Do not administer any other vaccines within 14 days before or after vaccination with ALPHA JECT 4000.
12. Do not vaccinate within 60 days prior to slaughter.
13. Ensure that the method of restraint, handling and administration minimizes the risk of accidental self injection. This product is a mineral oil-based compound. Accidental self injection may result in severe pain and swelling and requires prompt medical attention.

To the user:
This product contains mineral oil. Accidental injection/self injection may result in severe pain and swelling, particularly if injected into a joint or finger, and in rare cases could result in the loss of the affected finger if prompt medical attention is not given.

If you are accidentally injected with this product, seek prompt medical advice even if only a very small amount is injected and take the package leaflet with you.

If pain persists for more than 12 hours after medical examination, seek medical advice again.

PHARMAQ

30 August 2004

Product Information Sheet

Product name:	ALPHA JECT™ 4000 emulsion for injection

Manufacturer:	PHARMAQ AS, Skogmo Industriområde, N-7863 Overhalla, Norway

Composition:	
Active ingredients:	Formaldehyde inactivated cultures of the following bacteria: *Aeromonas salmonicida* subsp. *salmonicida* *Vibrio anguillarum* serotype O1 *Vibrio anguillarum* serotype O2 *Vibrio salmonicida*
Excipients:	Mineral oil and emulsifiers
Inactivation agent:	Residual free formaldehyde < 0.4 g/l
Preservative:	None

Maximum Residue Limits (MRL) Information:

In accordance with Article 1(2), Council Regulation (EEC) No. 2377/90 does not apply to active principles of biological origin intended to produce active or passive immunity used in veterinary immunological products. All other components (mineral oil, emulsifiers and formaldehyde) are included in Annex II to the Regulation (EEC) 2377/90, i.e. the substances are generally recognized as safe and no MRL is required.

Withdrawal Period:	Canada: 60 days Norway, United Kingdom: 0 days

Approvals/Markets:	Canada United Kingdom Norway

For any information about this veterinary medicinal product, please contact:

PHARMAQ AS, Harbitzalleen 5, P.O.Box 267 Skøyen, N-0213 Oslo, Norway

per PHARMAQ AS

Gina Galligani
Manager Regulatory Affairs

Advantigen 5.1

Injection vaccine for salmon

Contents
Formalin inactivated cultures of *Aeromonas salmonicida*, *Vibrio anguillarum* serotype 01 and 02, *Vibrio salmonicida*, *Moritella viscosa*

Manufacturer
Microtek International Inc., 6761 Kirkpatrick Crescent,
Saanichton, British Columbia, Canada, V0R 1L0.
Ph: 250.652.4482 Vet. Biol. Estab. Lic. No. 4

Indication
Active immunization of salmon against Furunculosis caused by *Aeromonas salmonicia*, Vibriosis caused by *Vibrio anguillarum* serotype 01 and 02 and Cold Water Vibriosis caused by *Vibrio salmonicida*, and for reduction of mortality caused by Winter Sores due to *Moritella viscosa*

Dosage and Administration
For fish ≥ 10g. Intraperitoneal injection with 0.1 mL per fish. The fish are to be sedated before the injection. The vaccination equipment shall be sterile before use. Shake the container well before use.

Contraindications
Vaccination shall only be performed on healthy fish.
Fish shall not be vaccinated at temperatures lower than 1°C.
Shall not be used if there are any signs of disease amongst the fish.

Withdrawal time
21 days. Fish butchered within 6 weeks after vaccination should be gutted.

Adverse reactions
Reduced appetite may be observed after vaccination.
Local inflammation reactions in the abdominal cavity may cause coalescence and or pigmentation spots.

Precautions
Use entire contents when first opened

Specific warnings
Only healthy fish to be vaccinated. Shall not be used together with other treatment or medication that may have an immunosuppressive effect.

Precautions (for persons handling the preparation)
Self injection in humans must be avoided. Protective equipment should be used (needle guard, gloves), also automatic injection equipment may be used. If a person inadvertently vaccinates himself one must immediately contact the nearest surgery/hospital and present the package insert that has instructions for the physician.

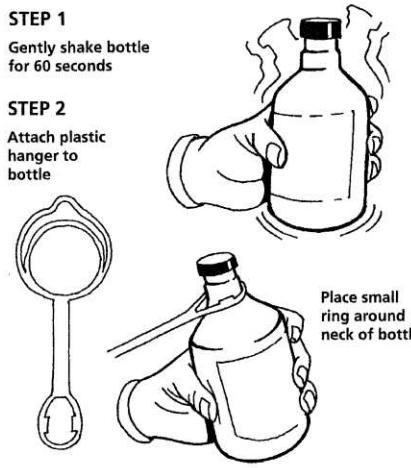
Information for the physician:
Inadvertent injection with oil based vaccines may cause vascular spasms. In particular if a fingertip or tendon is involved it may be necessary to perform a rapid incision and thorough lavage of the injected area. Normally pain reliving treatment and/or anti inflammatory preparations will be sufficient.

Durability
Stable until the date given on the label. To be stored from 2-7°C.
Do not freeze. Use the entire contents once the bottle has been opened

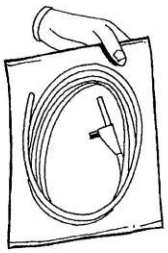
Package Specifications
500 mL polyethelene bottle, tube and spike

STEP 1
Gently shake bottle for 60 seconds

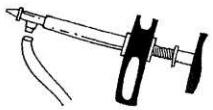
STEP 2
Attach plastic hanger to bottle



STEP 3
Remove the tubing with spike from the plastic bag



STEP 4
Connect the free end of the tubing to the injection gun



STEP 5
Remove the sheath from the spike end of the tubing and insert the spike into the rubber stopper in the centre of the bottle cap. Open the vent port located on the side of the spike to ensure proper venting of the bottle. Hang the bottle.

STEP 6
Attach a needle to the injection gun, pump the gun a few times to draw the vaccine to the gun and expel the air in the line

APPENDIX 5

Correlation matrices from the multilevel mixed model for each of the growth measures of individual Atlantic halibut collected over 4 time points for Weight (W), Length (L_F), specific growth rate (G) and proportional increase in weight (P_r), respectively with the type of correlation matrix specified.

W - Unstructured

	0	1	2	3	4
0	1				
1	0.91	1			
2	0.81	0.89	1		
3	0.77	0.9	0.95	1	
4	0.7	0.82	0.89	0.96	1

L_F -Unstructured

	0	1	2	3	4
0	1				
1	0.9	1			
2	0.75	0.86	1		
3	0.66	0.8	0.92	1	
4	0.63	0.77	0.9	0.85	1

G- Banded 2

	0	1	2	3	4
0	1				
1	0.16	1			
2	0.22	0	1		
3		0.39	-0.41	1	
4			0.06	0.29	1

P - Banded 2

	0	1	2	3	4
0	1				
1	0.14	1			
2	0.15	0.01	1		
3		0.37	-0.42	1	
4			0.13	0.31	1